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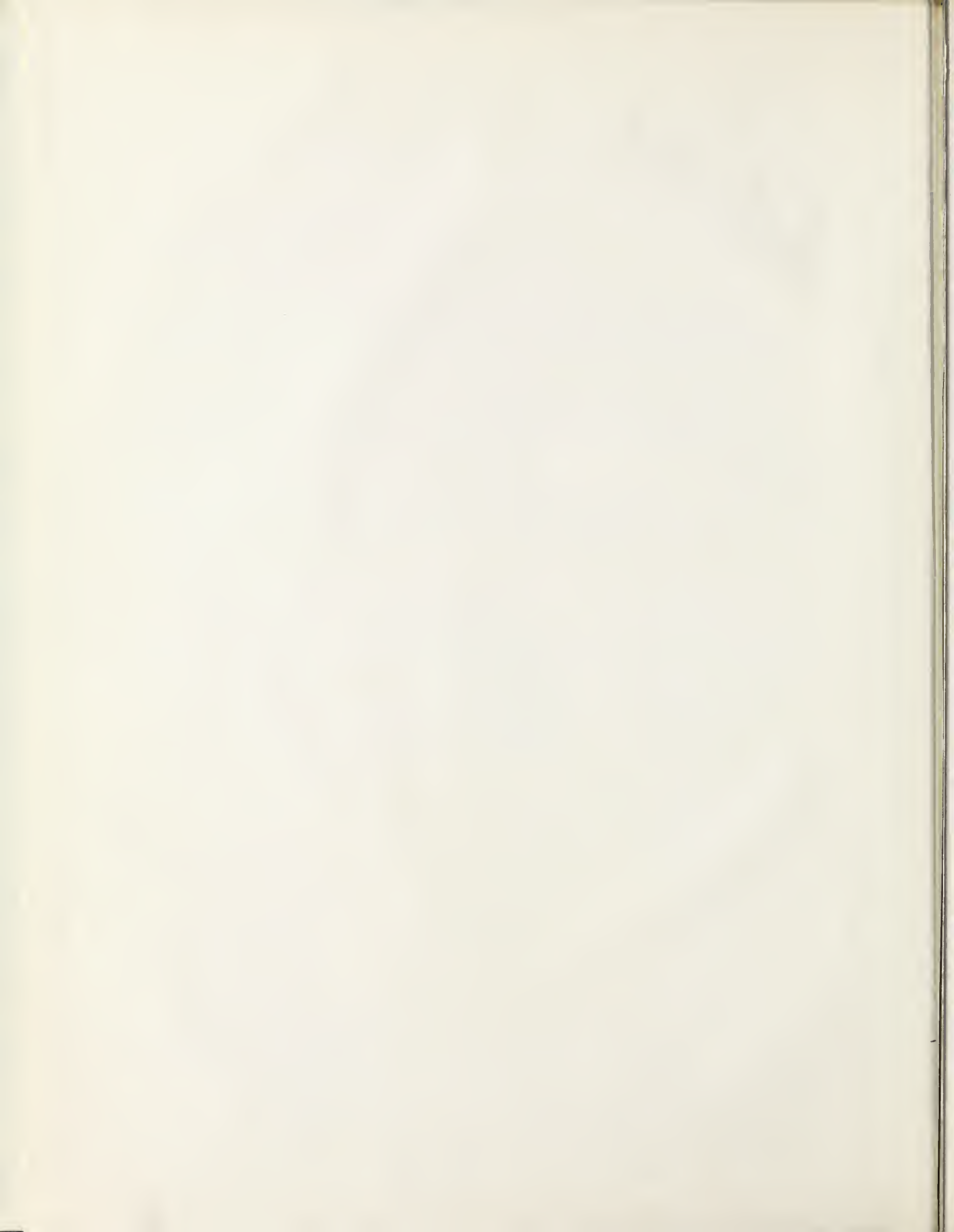


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THE EFFECTS OF GIBBERELLIC ACID ON GROWTH AND
FLOWERING OF Chrysanthemum morifolium,
Antirrhinum majus AND Dianthus caryophyllus

FACULTY OF GRADUATE STUDIES
DEPARTMENT OF PLANT SCIENCE
DIVISION OF HORTICULTURE

by

Te-sen Pih

EDMONTON, ALBERTA

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ABSTRACT

A majority of the reports available concerning the effects of gibberellic acid deal with studies of plants in natural day length and normal CO₂ conditions. Since the reports on the effects of gibberellic acid under controlled photoperiods and higher than normal CO₂ conditions are few, studies were initiated to investigate their effects on some common greenhouse flower crops. Experiments with Chrysanthemum morifolium, a short photoperiod plant were conducted to test the effects of GA₃ on morphological response as well as chlorophyll (a + b) content under different photoperiod regimes. Similar experiments with Antirrhinum majus and Dianthus caryophyllus were also conducted under normal and increased levels of carbon dioxide.

It was found that GA₃ increased the stem length and weight, leaf number and weight, and number of internodes both under long and short photoperiod regimes. The length of the first lateral peduncle and the diameter and weight of terminal inflorescences was increased under the short photoperiod regime. The stem strength, chlorophyll content and root weight of chrysanthemum plants growing in both short and long photoperiods were decreased as a result of GA₃ treatment.

In the studies with plants of Antirrhinum majus, it was found that GA₃ was effective in increasing the stem length, inflorescence (spike) length and top fresh weight both in CO₂ enriched and natural atmospheres. The chlorophyll content in

leaves and root length of snapdragon plant both in CO₂ enriched and natural atmosphere were decreased as a result of GA₃ treatment. Effects were more pronounced under conditions of CO₂ enrichment.

In experiments with Dianthus caryophyllus plants, it was found that the GA₃ increased the stem length, diameter of terminal flowers, top fresh weight both in CO₂ enriched and natural atmospheres. The stem strength was decreased as a result of GA₃ treatment both in CO₂ enriched and natural atmosphere. As with Antirrhinum majus plants the effects of GA₃ were more pronounced under increased levels of CO₂.

ACKNOWLEDGEMENTS

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The writer also wishes to express his appreciation for the financial assistance provided by the University of Alberta which helped to make this study possible.

CHAPTER I

The first part of the book is devoted to a general survey of the history of the subject. It begins with a brief account of the early attempts to explain the origin of life, and then proceeds to a more detailed consideration of the various theories which have been advanced. The author then discusses the evidence in support of each of these theories, and finally arrives at his own conclusions. The second part of the book is devoted to a more detailed consideration of the various theories which have been advanced. The author then discusses the evidence in support of each of these theories, and finally arrives at his own conclusions.

The third part of the book is devoted to a more detailed consideration of the various theories which have been advanced. The author then discusses the evidence in support of each of these theories, and finally arrives at his own conclusions.

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Introduction

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19. The nineteenth part deals with the social security situation.
20. The twentieth part deals with the overall situation.

INTRODUCTION

Gibberellins, a group of naturally occurring growth regulators have aroused the interests of horticulturalists because of published reports about their spectacular effects on plants. Within the past 10 years plant physiologists and horticulturalists of the western world have moved into existing areas of research with these chemicals which had an oriental beginning over 40 years ago.

Gibberellins for horticultural use are promising because they produce some effects which can not be induced by any other chemical means. Some of the more striking potentials known are induction of cell elongation and division under many environmental conditions (53), induction of flowering of long day or biennial plants (9, 34, 58); increase in size of particular organs of the plants such as flowers, pedicles or fruits (12, 14, 20, 37, 56); the breaking of dormancy of many plants (3, 38, 57); the overcoming of genetic and physiological dwarfism (50, 53, 57, 58); the stimulation toward accumulation of dry matter (1, 9, 28, 40, 58) and a broadening of the environment (temperature range, photoperiod) in which a plant may be successfully grown (2, 10).

During the last two years greenhouse experiments were performed to investigate the influences of gibberellic acid on Chrysanthemum morifolium, Antirrhinum majus and Dianthus caryophyllus. The purpose of these studies was to determine the specific effects of the growth regulator on stem growth, stem strength, leaf growth, flower development, root growth and chlorophyll content of these particular crops.

REVIEW OF LITERATURE

A. The History of Gibberellins

The story of gibberellins started about 40 years ago. In Asian countries such as Japan and Taiwan the farmers often found their rice plants were attacked by a disease known as bakanae disease which decreased yields as much as 40% (28). Due to the economic importance of this disease a Japanese plant pathologist, Sawata (47, 48) started to investigate the problem. After careful observation he found that the most characteristic symptom of the disease was the appearance of tall thin plants, markedly overgrowing their uninfected neighbours, hence the name "bakane" or "foolish seedling", which is now generally in use. Affected plants are not only taller, but the internodes and leaf sheaths are longer, the leaves longer, narrower, and thinner and the angle the leaves form with the culm increased. Root growth and tillering are reduced, and the plants usually appear chlorotic (7, 9, 53, 58). In mild infections flowering may be two to three days earlier but the ears are smaller and the yield reduced. More severe infections lead to the formation of adventitious roots at the aerial nodes, stem curvature at the nodes, leaf curling and finally root rot and death before flowering. On microscopic examination the plant system is found to contain mycelium. It was originally thought the plants grew taller due to some stimulation from the mycelium. In the early 1920's, a young graduate of Chiba Horticultural College - Eiichi Kurosawa - came to

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Taiwan to work with Sawata at the Central Research Institute of the Taiwan Department of Agriculture. His problem was to work on methods of controlling the bakanae disease which, at that time, was causing severe rice loss on the island (Taiwan). In the course of his investigations, Kurasawa became interested in the unusual symptom of hyperelongation which characterized the disease. He undertook to determine the nature of the responsible agent and, if possible, to isolate it. In the summer of 1925 Kurasawa started his experimental work on this phase of the problem, and by the following year published in Japanese his now classical paper "Experimental studies on the secretion of Fusarium heterosporum on rice plants." Kurasawa showed that the sterile filtrates from the bakanae fungus gave marked growth stimulation in rice and grass. In this paper appeared the first photograph found in the literature which illustrates the stimulatory effect of the fungus secretion (53, 54).

After considerable effort, Yabuta, Sumiki and Hayashi finally isolated the active white powder substances and in 1935, Yabuta gave it the name of gibberellin on the basis of the scientific name of the perfect stage of the fungus, Gibberella fujikuroi. This was the first use of this term in the literature. Later they reported the isolation of crystalline gibberellin A and B. The studies on the chemical structure of gibberellin started at this time and it was at this time also that work outside of Japan began.

The first work on gibberellin outside the orient was conducted by J.E. Mitchell and C.R. Angel at Camp Detrick, Maryland. They cultured the fungus and produced a marked growth stimulation in beans using the fungal culture medium. Their work prompted a group at the U.S. Department of Agriculture headed by Dr. Stodola, to undertake the isolation and characterization of gibberellin. A few months later the I.C.I. (Imperial Chemical Industries, in Britain) group also started work on gibberellin with Dr. Brian in charge of the research program. Both the American and British work has resulted in the isolation of entirely new compounds. Named gibberellin X by the U.S.D.A. group and gibberellic acid by the I.C.I. team, the latter name has now been accepted by all involved. These two groups have confirmed the Japanese work and contributed their own findings as well. Since then a large number of papers about the effects of gibberellin on plants have been published. No plant hormones have excited as much botanical and horticultural interest as the gibberellins. New naturally occurring gibberellins continue to be characterized and the number has reached thirteen with more definitely in view (48).

B. Nomenclature

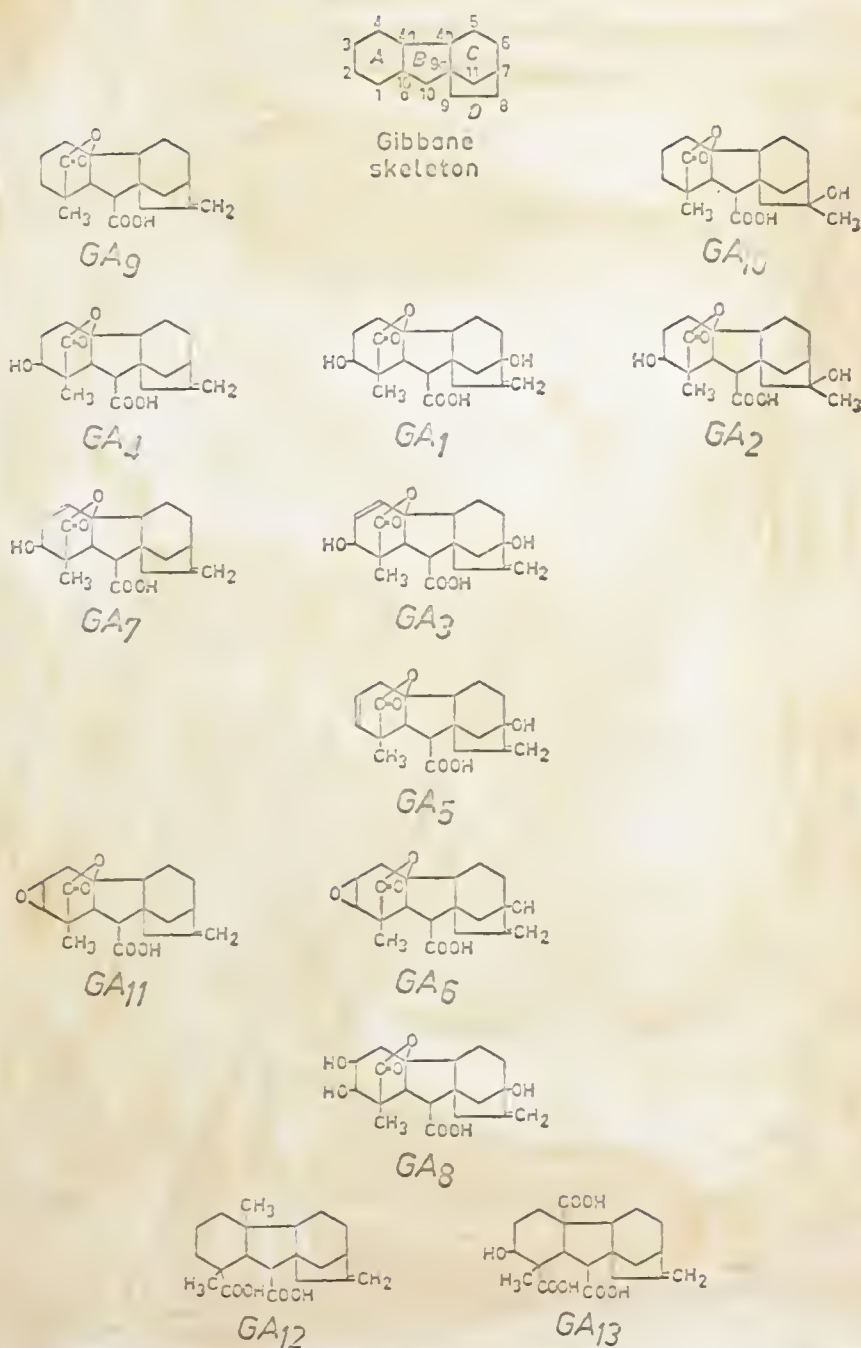
Biologically the active compounds of the gibberellin family can be divided into two classifications (48, 50).

1. Gibberellins

This term will be restricted to those substances that (1) possess the same carbon skeleton as gibberellic acid or one

very closely related to it, and

(2) are biological active in stimulating cell division and cell elongation, or both, in plants. This would restrict the use of the term gibberellin to compounds of the type currently named as gibberellin. In line with the current designations of compounds of this group, it has been suggested that a letter be adopted to indicate a particular carbon skeleton with a subscript number to indicate the specific compound with that carbon skeleton. Thus, the gibberellin A series would include the gibberellin A₁, gibberellin A₂ through GA₁₃, all of which have the same carbon skeleton, as follows:



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TO THE PRESENT
BY
JOHN STOW
1618



In this scheme gibberellic acid has the designation GA₃.

2. Gibberellin-like Substances

This term refers to those compounds with an unknown or different chemical configuration from gibberellic acid but the requisite biological activity in appropriate intact dwarf mutant tests. As suggested by Phinney and Went (50) the test should be carried out on mutants in which growth form is dependent on a single gene, and in which the growth response is specific for the gibberellins.

C. General Physiological and Morphological Effects of Gibberellin on Horticultural Plants

1. Elongation of Stems

The most typical response of plants to gibberellin is stem elongation. Elongation results primarily from an increase in the length of internodes (15), but sometimes from an increase in the number of nodes (11, 22). Usually only actively growing tissues respond (58) and the weight of these affected parts increases (1, 12, 28, 40, 53, 57, 52). Marth, et. al. (40) reported that:

- (a) With the petunia variety Purple Prince treated with gibberellic acid on the stem, a 61% increase in stem length over the control was obtained.
- (b) The response of newly rooted cuttings of Rhododendrum variety Roseum elegans treated with gibberellic acid on the stem varied from no effect to a marked 200% increase

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in stem length over controls.

- (c) With rose varieties, Better Times and Snow White treated with gibberellic acid on the stems, 51% and 34% increases in stem length respectively over controls were obtained.
- (d) Salvia (dwarf) treated with gibberellic acid on stems produced a 150% increase in stem length over controls.
- (e) Snapdragon treated with gibberellic acid on stems produced a 50% increase in stem length over controls.
- (f) Tulips (variety Seven-Top) treated with 100 ppm of gibberellic acid on stems resulted in a 20% increase in stem length over controls, but lower concentrations had no effect on stem length.
- (g) Hydrangea (variety Todi) treated with gibberellic acid on stems resulted in a 100% increase in stem length over controls.

Cathey (14) reported that the stem of Chrysanthemum morifolium was responsive to applications of aqueous sprays of a gibberellic acid at any time during growth, but application during long photoperiod was not as effective as treatment during short photoperiod.

2. Induction of Flowering

Prior to the discovery and use of gibberellin only a few plants had been induced to flower by chemical treatment (15). Gibberellin has been shown to replace the cold treatment necessary for the flowering of some biennials and the long day requirement of some annuals (3, 10, 34, 53, 58). Most species which produce rosette plants in the garden; e.g., column stock, foxglove

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DEPARTMENT OF CHEMISTRY
530 CHICAGO HALL
CHICAGO, ILL. 60637
U.S.A.
TEL: (312) 837-3000
FAX: (312) 837-3000
E-MAIL: chem@uchicago.edu
WWW: <http://www.uchicago.edu/chem>

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DEPARTMENT OF CHEMISTRY
530 CHICAGO HALL
CHICAGO, ILL. 60637
U.S.A.
TEL: (312) 837-3000
FAX: (312) 837-3000
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FAX: (312) 837-3000
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and larkspur, may be flowered the first year from seeds by treatment with gibberellin (37). More than one treatment with gibberellin, however, may be necessary to make these plants flower as annuals (15). Marth, et. al. (40) reported that:

- (a) Plants of petunia variety, Purple Prince, treated with gibberellic acid on stems blossomed more profusely and 10 days earlier than untreated ones.
- (b) Salvia (dwarf) treated with gibberellic acid blossomed 7 to 10 days earlier.
- (c) Dahlia treated with gibberellic acid on stems flowered 7 to 10 days earlier.

Cathey (14) reported that when a concentrated solution of gibberellin (1000 ppm) was applied to Chrysanthemum morifolium when the outer florets of the inflorescence were showing color, the florets developed faster than did those of untreated plants.

3. Organ Size and Shape

The size of peduncles, pedicles, and petals are affected by treatment with gibberellin (15, 20, 37, 42). Geraniums sprayed with a 10 ppm solution of gibberellin when flowers were showing color improved in keeping quality and petal size as well as in length of pedicels (37). It has been reported by Marth, et. al. (40) that Antirrhinum majus treated with 1% lanolin of gibberellic acid developed many branches from the nodes with no branches on control plants. Hydrangea macrophylla (42), after cool storage developed larger sepals when the plants were sprayed four times with a 10 ppm solution as compared with unsprayed plants.

Application of five times that amount of gibberellin (50 ppm) damaged the leaves and sepals, the resulting plants being excessively elongated with lanceolate leaves. Some mature branches of Hedera helix became completely juvenile when the plants were treated with gibberellin (52). Reversion from juvenile to the mature type of growth is a constant problem in the maintenance of certain plant specimens. Many ornamental plants are most desirable when they are in juvenile growth. The evidence from Hedera helix indicates a possibility that gibberellin, when properly used, may aid in maintaining this type of growth. Such a response in many woody plants, however, would be undesirable because juvenile growth is an undesirable and unproductive phase in their growth.

4. Root Growth

Although bakanae disease increases adventitious root formation (53) there is general agreement that underground root growth is reduced in length and weight (1, 15, 28, 29). The Tokyo group of workers generally found inhibition or else no effect. The I.C.I. workers (9) have obtained consistent root weight decrease in treated peas and wheat. They point out that nearly all reports are of plants treated via nutrient solutions which could result in local root inhibitions. Treatment of seedlings with concentrated solutions or frequent applications of gibberellins reduced the root growth of yellow birch, Betula alleghaniensis (35). Some reports have indicated that gibberellin B (allogibberic acid) (47) promotes root growth. The overall effects of gibberellin when it stimulates the shoot and

inhibits the root is to cause a marked change in the ratio of shoot weight to root weight. This may indicate an actual redistribution of material within the plant from root to shoot (6), but whether this is always true is still uncertain.

5. Dry Matter Accumulation

Marth, et. al. reported (40) that the dry weight of peas and soybeans increased with treatment by gibberellin. Brian, et. al. (6) indicated an increase over controls in the dry weight of treated wheat plants. Hayashi (28) reported that the top dry weight of tomato plants increased about 18% as a result of treatment with gibberellin but did not increase in dry weight on a unit leaf area basis (23). The dry weight of stems of Chrysanthemum morifolium treated with gibberellin was reported highly increased but the fresh and dry weight of leaves was only slightly affected (14). Such dry weight increases reflect an increase in carbon assimilation since mineral constituents represent only a very small portion of total dry weight. Since the efficiency of the photosynthetic process is not apparently affected (23), it seems most probable that the dry weight is the indirect response of treatment with gibberellins (9). Even when shoot dry weight is increased by gibberellin treatment, root dry weight is usually reduced (1, 15, 28, 29). There is some evidence that, as a result of gibberellin treatment, the ratio of dry weight of shoot to dry weight of root changes, a disproportionate part of the extra carbon assimilated being incorporated in shoot tissue (6, 9).

The first part of the paper is devoted to a general discussion of the problem of the existence of solutions of the system of equations (1) for arbitrary values of the parameters α and β . It is shown that the system has solutions for all values of the parameters α and β if and only if the condition $\alpha + \beta > 1$ is satisfied. In the case when this condition is not satisfied, the system has no solutions.

In the second part of the paper, the problem of the uniqueness of solutions of the system (1) is considered. It is shown that the system has a unique solution for all values of the parameters α and β if and only if the condition $\alpha + \beta > 1$ is satisfied. In the case when this condition is not satisfied, the system has no solutions.

In the third part of the paper, the problem of the stability of solutions of the system (1) is considered. It is shown that the system has stable solutions for all values of the parameters α and β if and only if the condition $\alpha + \beta > 1$ is satisfied. In the case when this condition is not satisfied, the system has unstable solutions.

In the fourth part of the paper, the problem of the asymptotic behavior of solutions of the system (1) is considered. It is shown that the system has asymptotically stable solutions for all values of the parameters α and β if and only if the condition $\alpha + \beta > 1$ is satisfied. In the case when this condition is not satisfied, the system has asymptotically unstable solutions.

In the fifth part of the paper, the problem of the bifurcation of solutions of the system (1) is considered. It is shown that the system has bifurcating solutions for all values of the parameters α and β if and only if the condition $\alpha + \beta > 1$ is satisfied. In the case when this condition is not satisfied, the system has no bifurcating solutions.

In the sixth part of the paper, the problem of the global existence of solutions of the system (1) is considered. It is shown that the system has globally existing solutions for all values of the parameters α and β if and only if the condition $\alpha + \beta > 1$ is satisfied. In the case when this condition is not satisfied, the system has no globally existing solutions.

In the seventh part of the paper, the problem of the periodicity of solutions of the system (1) is considered. It is shown that the system has periodic solutions for all values of the parameters α and β if and only if the condition $\alpha + \beta > 1$ is satisfied. In the case when this condition is not satisfied, the system has no periodic solutions.

In the eighth part of the paper, the problem of the ergodicity of solutions of the system (1) is considered. It is shown that the system has ergodic solutions for all values of the parameters α and β if and only if the condition $\alpha + \beta > 1$ is satisfied. In the case when this condition is not satisfied, the system has no ergodic solutions.

In the ninth part of the paper, the problem of the mixing of solutions of the system (1) is considered. It is shown that the system has mixing solutions for all values of the parameters α and β if and only if the condition $\alpha + \beta > 1$ is satisfied. In the case when this condition is not satisfied, the system has no mixing solutions.

In the tenth part of the paper, the problem of the transitivity of solutions of the system (1) is considered. It is shown that the system has transitive solutions for all values of the parameters α and β if and only if the condition $\alpha + \beta > 1$ is satisfied. In the case when this condition is not satisfied, the system has no transitive solutions.

6. Chlorosis

The typical yellowing symptom of the bakanae disease is also brought about by gibberellin treatment (7, 9, 53, 60, 61). Fewer chloroplasts are present in diseased tissue and the total chlorophyll content decreases. Increased plant growth brought about by gibberellin is generally accompanied by a paling of the leaves (7, 61). This leaf paling can be reduced to a considerable extent by ensuring that mineral nutrients, especially nitrogen, are not limiting, but even under the best nutrient conditions some chlorosis is usually visible (9). Wolf and Haber (60) recently reported that the chlorosis does not necessarily accompany growth stimulation by gibberellin. The paling in very young seedlings results solely from a failure of chlorophyll synthesis to keep pace with the increased cell expansion. Besides this simple dilution of chlorophyll, the apparent chlorosis in old plants must also result from the delayed effect of malnutrition, not to direct action of the gibberellin on formation or destruction of chlorophyll.

7. Other Responses of Plants to Gibberellins

In addition to the above mentioned responses of plants to gibberellins, other interesting and promising responses which have been reported are:

- (a) the modification of sex expression in cucumber plants (13, 43, 49, 58, 59);
- (b) the breaking of dormancy (3, 15, 19, 38, 50, 51, 58);
- (c) the increasing of enzyme activity (19, 25);
- (d) the reversing of dwarfism (48, 50, 53, 58);

- (e) the broadening of environments (temperature, photoperiod) in which a plant may be successfully grown (2, 4, 10, 15, 22, 30, 34, 50, 53, 58);
- (f) the increasing of reducing sugar content (19, 28, 58) and cellulose (57, 58) and the lowering of starch content (28);
- (g) the induction of parthenocarpy (50);
- (h) the increasing of auxin content after gibberellin treatment (48);
- (i) the increasing of respiration rates (19, 25, 46, 53, 57); and
- (j) the reversing of inhibition by growth-retarding chemicals (16, 22, 45, 57, 58).

III. THE EFFECTS OF GA₃ ON GROWTH AND FLOWERING OF Chrysanthemum morifolium PLANTS GROWING UNDER LONG AND SHORT PHOTOPERIODS

Gibberellic acid is effective in accelerating the elongation of stems (11, 20, 40), nullifying the cold requirement of rosetting plants (34) and eliminating the light requirement for the germination of lettuce seeds (30). These known effects suggest that gibberellic acid should increase the height and accelerate the flowering of plants in which the development is controlled by photoperiod. Therefore, the effect of the time of application of gibberellic acid on growth and flowering of Chrysanthemum morifolium, a short day plant, were studied.

Investigations conducted in the university greenhouses during the summer months of 1965 concerned the responses of Chrysanthemum morifolium to gibberellic acid. The responses studied included the effect of this substance on the stem length, stem strength, stem weight (terminal stem without leaves), internode number, leaf number and weight, terminal inflorescence size and weight, root weight and dry weight of stems, leaves and inflorescences.

Materials and Methods

Experimental work was conducted in the greenhouse of the Horticulture, Division, the University of Alberta, Edmonton, Alberta. General commercial cultural methods as outlined by Laurie and Kiplinger (36) were followed except where specific treatments were involved.

Rooted cuttings of the variety Iceberg were obtained from Yoder Brothers Inc., Barberton, Ohio, U.S.A. The cuttings were benched in a steamed sterilized soil mixture (3 soil, 2 sand and 1 peat) at a spacing of 8 x 8 inches on June 28, 1965. Long photoperiod was maintained under the natural summer day length. Short photoperiod was produced by covering the plants with black sateen cloth at 4:30 p.m. and removing it at 8:30 a.m. each day. Long photoperiod was continued for all plants until the plants became well established. All plants were pinched on July 15, 1965. The short photoperiod (8-hour) treatment was started on July 19, 1965, and ended when the flower buds began to show color. The plants were sprayed with aqueous mixtures of gibberellic acid prepared by dissolving the required amount of the chemical in a minimum of ethanol, mixing sufficient Tween 20 to produce a 0.1 percent solution and diluting to the desired concentrations with distilled water. The aqueous solutions were stored at 50° F for up to 5 days. Unused portions were discarded after this time. The apical region and the expanding leaves of the plant were sprayed with a hand atomizer until the excess solution began to run off. Approximately 1 ml of solution was required per plant per application. Twice weekly applications were made for a total of six applications to each treated plant both in long and short photoperiod regimes.

The GA₃ chemical used in these experiments was a product of Eastman Kodak Company (85% gibberellic acid). In these

studies the gibberellic acid was applied as a foliar spray treatment only.

The experimental design used in these experiments was a randomized block design with five treatments replicated four times. Each treatment plot contained 5 plants. The outside rows of plants along the edge of the benches served as guard rows.

The concentrations used were as follows:

<u>Treatment</u>	<u>Concentration</u>
1	0 ppm
2	5 ppm
3	10 ppm
4	50 ppm
5	100 ppm

1. Chlorophyll Determination

Two weeks after the last treatment application of GA₃ was made (September 5, 1965) leaf samples were taken from each treated plant and the composite samples thoroughly mixed for each treatment replication. From these mixtures 300 mg samples were weighed out for chlorophyll analysis to ascertain if chlorophyll levels could be influenced by treatment with gibberellic acid.

Each 300 mg fresh sample was ground in a mortar and the chlorophyll extracted with 80% reagent grade acetone. The extract solutions were filtered through two layers of Whatman's No. 1 filter paper and made up to 50 ml volume with acetone. If the filtered extract could not be assayed immediately it was stored

The first part of the paper is devoted to a general discussion of the problem of the existence of solutions of the system of equations (1) for arbitrary values of the parameters α and β . It is shown that the system has solutions for all values of the parameters α and β if the function $f(x)$ is continuous and has a bounded derivative. The second part of the paper is devoted to a detailed study of the properties of the solutions of the system (1) for arbitrary values of the parameters α and β . It is shown that the solutions of the system (1) are unique and depend continuously on the parameters α and β . The third part of the paper is devoted to a study of the asymptotic properties of the solutions of the system (1) for large values of the parameters α and β . It is shown that the solutions of the system (1) approach zero as the parameters α and β approach infinity.

α	β	x	y
1.0	1.0	0.0	0.0
1.0	1.0	0.1	0.1
1.0	1.0	0.2	0.2
1.0	1.0	0.3	0.3
1.0	1.0	0.4	0.4
1.0	1.0	0.5	0.5
1.0	1.0	0.6	0.6
1.0	1.0	0.7	0.7
1.0	1.0	0.8	0.8
1.0	1.0	0.9	0.9
1.0	1.0	1.0	1.0

The fourth part of the paper is devoted to a study of the asymptotic properties of the solutions of the system (1) for small values of the parameters α and β . It is shown that the solutions of the system (1) approach zero as the parameters α and β approach zero. The fifth part of the paper is devoted to a study of the asymptotic properties of the solutions of the system (1) for large values of the parameters α and β . It is shown that the solutions of the system (1) approach zero as the parameters α and β approach infinity. The sixth part of the paper is devoted to a study of the asymptotic properties of the solutions of the system (1) for small values of the parameters α and β . It is shown that the solutions of the system (1) approach zero as the parameters α and β approach zero.

The seventh part of the paper is devoted to a study of the asymptotic properties of the solutions of the system (1) for large values of the parameters α and β . It is shown that the solutions of the system (1) approach zero as the parameters α and β approach infinity. The eighth part of the paper is devoted to a study of the asymptotic properties of the solutions of the system (1) for small values of the parameters α and β . It is shown that the solutions of the system (1) approach zero as the parameters α and β approach zero. The ninth part of the paper is devoted to a study of the asymptotic properties of the solutions of the system (1) for large values of the parameters α and β . It is shown that the solutions of the system (1) approach zero as the parameters α and β approach infinity.

in a freezer. A Beckman DK1 recording spectrophotometer was used to determine absorption readings. Two one ml samples of each extract solution were pipetted into 1 cm sq. quartz cuvettes and absorbance reading measured over the light wavelength of 663 to 645 millimicrons (μ) and averaged to obtain absorbance values for each treatment. These average values were then converted to mg chlorophyll per mg fresh weight by the use of the conversion formulae (62):

$$\text{Chlorophyll a} = (0.01230 D_{663} - 0.00086 D_{645}) V/W$$

$$\text{Chlorophyll b} = (0.0193 D_{645} - 0.0036 D_{663}) V/W$$

where D = density or absorbancy

V = volume

W = weight of sample

2. General Measurement Determination

The plants were harvested on September 23 to September 27, 1965. Measurements were taken of the stem length (from the tip to the bottom of terminal stem), stem weight (whole terminal stem without leaves), internode number, leaf number and weight, terminal inflorescence size and weight as well as root weight.

3. Stem Strength Determination

A tenderometer was used to determine stem strength and the method was briefly as follows:

- (a) The upper 50 cm of the terminal shoot of each test plant were removed for stem strength determination.
- (b) Only the basal portion (26 cm) of these 50 cm stem

The first part of the paper is devoted to a discussion of the
general principles of the theory of the structure of the
crystal lattice. It is shown that the structure of the
crystal lattice is determined by the arrangement of the
atoms in space. The arrangement of the atoms is
determined by the forces of attraction and repulsion
between them. The forces of attraction are due to the
electrostatic interaction between the positive and
negative ions. The forces of repulsion are due to the
Pauli exclusion principle.

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properties of the crystal lattice. It is shown that the
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electrostatic interaction between the positive and
negative ions. The forces of repulsion are due to the
Pauli exclusion principle.

segments was used for stem strength determination since the upper portions were immature and very tender.

- (c) The portion of each stem chosen for analysis was cut into four equal parts and each part analyzed separately by the tenderometer. The resistance offered by the stem segment to the shearing action of the machine was recorded in pounds of pressure.
- (d) The overall stem strength readings recorded as lb/stem were the average of the four sections per stem.

4. Dry Weight Determination

The dry weight of leaves, stems and inflorescences was obtained by drying fresh samples for 24 hours in an oven maintained at 60° C. Five samples were run for each treatment replication and the average taken.

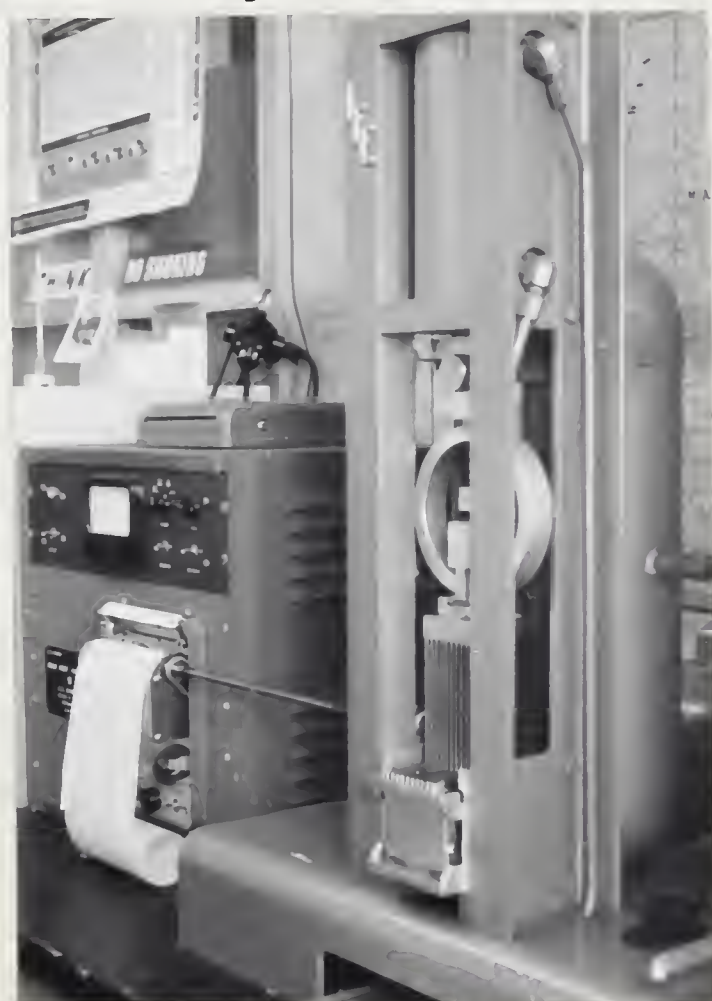


Figure 2. Tenderometer used in experiments to determine stem strength.

RESULTS

Three days after the first GA₃ application, plants appeared to grow more rapidly, especially those treated with high concentrations. This became more apparent after the third GA₃ application. Plants which received 50 and 100 ppm appeared slightly paler green. After the sixth GA₃ application, the terminal stem trended to bend downward.

A. Effects of GA₃ on the Stem Length of Chrysanthemum Plants Grown under Short and Long Photoperiod Regimes

GA₃ applications significantly increased the stem length of chrysanthemum plants compared with control plants. The stem lengths were recorded at harvest time for each treatment under both short and long photoperiods. Analysis of variance in Table I indicated highly significant differences among the treatments under both long and short photoperiods. Duncan's Multiple Range Test also showed significant differences among treatments. The differences in stem length are summarized in Table I.

B. Effects of GA₃ on the Stem Strength of Chrysanthemum Plants Under Short and Long Photoperiod Regimes

The stem strength was influenced to a significant degree as is shown by the figures in Table II. Analysis of variance produced a highly significant F value. Duncan's Multiple Range Test revealed that there were significant differences among treatments under both short and long photoperiod regimes. The differences in stem strength are summarized in Table II.



Figure 3. The effect of GA_3 on the stem and the 1st lateral peduncle length of Chrysanthemum morifolium (Iceberg) plants growing under short photoperiod. The concentrations used from left to right are 0, 5, 10, 50 and 100 ppm.



Figure 4. The effect of GA_3 on the stem length of Chrysanthemum morifolium (Iceberg) plants growing under long photoperiod. The concentrations used from left to right are 0, 5, 10, 50 and 100 ppm.

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TABLE I

The influence of GA₃ on the stem length of Chrysanthemum mori-
folium (Iceberg) plants growing under
short and long photoperiod regimes

A. Mean Stem Length

Treatments	Mean stem length in cm	
	Short Day	Long Day
0 ppm GA ₃	70.50 ^d	74.50 ^d
5 ppm GA ₃	91.29 ^c	86.45 ^c
10 ppm GA ₃	99.59 ^b	90.05 ^c
50 ppm GA ₃	110.56 ^a	99.05 ^b
100 ppm GA ₃	113.31 ^a	106.26 ^a

B. Analysis of Variance

Source	DF	MS	Mean Stem Strength		
			Short Day F	MS	Long Day F
Replications	3				
Treatments	4	1254.34	97.97**	583.38	39.15**
Error	12	12.81		14.90	

**Significant at 1% level.

Numbers in each column that are not followed by the same letter are significant from each other at the 5% level of significance as judged by Duncan's New Multiple Range Test.

TABLE II

The influence of GA₃ on the stem strength of Chrysanthemum morifolium (Iceberg) plants growing under short and long photoperiod regimes

A. Mean Stem Strength

Treatments	Mean stem strength (lb/stem)	
	Short Day	Long Day
0 ppm GA ₃	541.7 ^a	1262.5 ^a
5 ppm GA ₃	465.1 ^b	1042.5 ^a
10 ppm GA ₃	486.5 ^b	1038.7 ^b
50 ppm GA ₃	446.7 ^b	771.3 ^b
100 ppm GA ₃	326.5 ^c	626.3 ^c

B. Analysis of Variance

Source	DF	MS	Mean Stem Strength	
			Short Day F	Long Day F
Replications	3			
Treatments	4	24633.06	48.47**	250839.38 17.09**
Error	12	508.20		14675.21

**Significant at 1% level.

Numbers in each column that are not followed by the same letter are significant from each other at the 5% level of significance as judged by Duncan's New Multiple Range Test.

Table 1

Summary of the results of the experiments conducted on the effect of the concentration of the solution on the rate of reaction.

Concentration of solution (M)

Concentration of solution (M)	Rate of reaction (M/s)	Time taken (s)
0.1	0.001	100
0.2	0.002	50
0.3	0.003	33
0.4	0.004	25
0.5	0.005	20

Rate of reaction (M/s)



The graph shows a direct proportionality between the concentration of the solution and the rate of reaction. As the concentration increases, the rate of reaction also increases linearly.

C. Effects of GA₃ on the Terminal Stem Fresh Weight of Chrysanthemum plants Growing under Short and Long Photoperiod Regimes

The stem fresh weight was increased as a result of GA₃ treatment. Analysis of variance indicated that the F value did not exceed the 5% level of significance. Duncan's Multiple Range Test revealed that there were significant differences among treatments both under short and long photoperiod regimes. The differences in fresh weight of terminal stems are summarized in Table III.

D. Effects of GA₃ on the Number of Internodes of Terminal Stems of Chrysanthemum Plants Growing Under Short and Long Photoperiod Regimes

GA₃ applications significantly increased the number of internodes of chrysanthemum plants compared to controls. The number of internodes were recorded at harvest time for each treatment under both short and long photoperiods. In Table IV is evidence that the long photoperiod increased the internodes number more than did the short photoperiod regime. Analysis of variance showed that both F values exceeded the 5% level of significance. Duncan's Multiple Range Test indicated that there were significant differences among treatments. The differences in the number of internodes are summarized in Table IV.

E. Effects of GA₃ on the Leaf Number of Terminal Stem of Chrysanthemum Plants Growing under Short and Long Photoperiod Regimes

TABLE III

The influence of GA₃ on the terminal stem fresh weight of Chrysanthemum morifolium (Iceberg) plants growing under short and long photoperiod regimes

A. Mean Stem Fresh Weight (without leaf)

Treatments	Mean Stem Weight in Grams	
	Short Day	Long Day
0 ppm GA ₃	12.40 b	9.10 b
5 ppm GA ₃	13.24 b	12.50 ^{ab}
10 ppm GA ₃	14.65 ^{ab}	12.85 ^{ab}
50 ppm GA ₃	14.08 ^{ab}	12.73 ^{ab}
100 ppm GA ₃	16.55 ^a	14.28 ^a

B. Analysis of Variance

Source	DF	MS	Mean Stem Weight		
			Short Day F	MS	Long Day F
Replications	3				
Treatments	4	9.929	2.567 ^{N.S.}	14.603	2.09 ^{N.S.}
Error	12	3.868		7.015	

Numbers in each column that are not followed by the same letter are significant from each other at the 5% level of significance as judged by Duncan's New Multiple Range Test.

N.S. Does not exceed the 5% level of significance.

It is hereby certified that the foregoing is a true and correct copy of the original as the same appears in the records of the Department of the Interior.

Wm. H. Hunt, Secretary of the Interior.

STATE OF CALIFORNIA.

County.	Assessor.	Amount.
Alameda	John A. Smith	\$100,000.00
Butte	John A. Smith	\$100,000.00
Colusa	John A. Smith	\$100,000.00
Contra Costa	John A. Smith	\$100,000.00
El Dorado	John A. Smith	\$100,000.00

Witness my hand and seal of office this 1st day of January, 1900.

STATE OF CALIFORNIA.

County.	Assessor.	Amount.
Alameda	John A. Smith	\$100,000.00
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It is hereby certified that the foregoing is a true and correct copy of the original as the same appears in the records of the Department of the Interior.

TABLE IV

The influence of GA₃ on the number of internodes of terminal stem of Chrysanthemum morifolium (Iceberg) plants growing under short and long photoperiods regimes

A. Mean Number of Internodes

Treatments	Mean Internodes Number	
	Short Day	Long Day
0 ppm GA ₃	23.075 b	18.25 c
5 ppm GA ₃	24.025 ^{ab}	20.75 b
10 ppm GA ₃	24.300 ^{ab}	21.00 b
50 ppm GA ₃	24.800 ^a	23.25 ^a
100 ppm GA ₃	25.050 ^a	24.50 ^a

B. Analysis of Variance

Source	DF	MS	Mean Internodes Number		
			Short Day F	MS	Long Day F
Replications	3				
Treatments	4	2.376	3.459*	23.425	13.845**
Error	12	0.687		1.692	

*Significant at 5% level.

**Significant at 1% level.

Numbers in each column that are not followed by the same letter are significant from each other at the 5% level of significance as judged by Duncan's New Multiple Range Test.

The leaf number was increased as a result of treatment with GA₃. Analysis of variance revealed that both F values exceeded the 5% level of significance. Duncan's Multiple Range Test indicated that there were significant differences among treatments both under short and long photoperiod regimes. The differences in leaf number are summarized in Table V.

F. Effects of GA₃ on the Leaf Fresh Weight of Terminal Stem Leaves of Chrysanthemum Plants Growing under Long and Short Photoperiod Regimes

The average weight of leaves on the terminal stems was increased with GA₃ treatment. Analysis of variance indicated that both F values did not exceed the 5% level of significance. Duncan's Multiple Range Test revealed that there were significant differences among treatments only in short photoperiod regimes. The differences in leaf fresh weight are summarized in Table VI.

G. Effects of GA₃ on the Root Weight of Chrysanthemum Plants Growing under Short and Long Photoperiod Regimes

GA₃ applications significantly decreased the root weight of chrysanthemum plants compared with control. Analysis of variance indicated that both F values exceeded the 1% level of significance. Duncan's Multiple Range Test showed that no significant differences existed among the treatments but all treatments varied significantly from the control both under short and long photoperiod regimes (Table VII).

TABLE V

The Influence of GA₃ on the leaf number of terminal stem of Chrysanthemum morifolium (Iceberg) plants growing under long and short photoperiod regimes

A. Mean Leaf Number

Treatments	Mean Leaf Number	
	Short Day	Long Day
0 ppm GA ₃	21.15 ^b	16.75 ^c
5 ppm GA ₃	22.46 ^a	20.00 ^{bc}
10 ppm GA ₃	22.93 ^a	20.75 ^{bc}
50 ppm GA ₃	23.10 ^a	21.25 ^{ab}
100 ppm GA ₃	22.90 ^a	22.75 ^a

B. Analysis of Variance

Source	DF	MS	Mean Leaf Number		
			Short Day F	MS	Long Day F
Replications	3				
Treatments	4	2.523	3.936*	19.55	7.767**
Error	12	0.641		0.517	

*Significant at 5% level.

**Significant at 1% level.

Numbers in each column that are not followed by the same letter are significant from each other at the 5% level of significance as judged by Duncan's New Multiple Range Test.

TABLE VI

The influence of GA₃ on the leaf fresh weight of terminal stem leaves of Chrysanthemum morifolium (Iceberg) plants growing under long and short photoperiod regimes

A. Mean Leaf Weight

Treatments	Mean Leaf Weight in Grams	
	Short Day	Long Day
0 ppm GA ₃	15.75 b	15.18 ^a
5 ppm GA ₃	16.80 ^{ab}	17.08 ^a
10 ppm GA ₃	17.35 ^{ab}	17.00 ^a
50 ppm GA ₃	19.15 ^a	16.15 ^a
100 ppm GA ₃	19.92 ^a	17.38 ^a

B. Analysis of Variance

Source	DF	MS	Mean Leaf Weight		
			Short Day F	MS	Long Day F
Replications	3				
Treatments	4	11.753	3.126 ^{N.S.}	8.171	0.948 ^{N.S.}
Error	12	3.757		8.620	

N.S. Does not exceed the 5% level of significance.

Numbers in each column that are not followed by the same letter are significant from each other at 5% level of significance as judged by Duncan's New Multiple Range Test.

TABLE VII

The influence of GA₃ on the root weight of Chrysanthemum morifolium (Iceberg) plants growing under short and long photoperiod regimes

A. Mean Root Weight

Treatments	Mean Root Weight in Grams	
	Short Day	Long Day
0 ppm GA ₃	19.35 ^b	18.66 ^b
5 ppm GA ₃	13.20 ^a	14.01 ^a
10 ppm GA ₃	13.07 ^a	13.29 ^a
50 ppm GA ₃	13.50 ^a	12.84 ^a
100 ppm GA ₃	12.80 ^a	12.42 ^a

B. Analysis of Variance

Source	DF	MS	Mean Root Weight		
			Short Day F	MS	Long Day F
Replications	3				
Treatments	4	14.383	6.066**	25.781	90.83**
Error	12	2.371		0.263	

**Significant at 1% level.

Numbers in each column that are not followed by the same letter are significant from each other at the 5% level of significance as judged by Duncan's New Multiple Range Test.

H. Effects of GA₃ on the Dry Weight of Terminal Stems of Chrysanthemum Plants Growing under Short and Long Photoperiod Regimes

Generally speaking, the average dry weight of terminal stems was increased as a result of GA₃ treatment. Duncan's Multiple Range Test indicated that there were significant differences among treatments. The differences in dry weight of terminal stems are summarized in Table VIII.

I. Effects of GA₃ on the Dry Weight of Leaves of Chrysanthemum Plants Growing under Short and Long Photoperiod Regimes

The dry weight of leaves was influenced by treatment with GA₃. Duncan's Multiple Range Test revealed that there were significant differences among treatments under both short and long photoperiod regimes. The differences in dry weight of leaves on terminal stems are summarized in Table IX.

J. Effects of GA₃ on the Weight and Diameter of Terminal Inflorescences of Chrysanthemum Plants Growing Under Short Photoperiod Regime

The application of GA₃ significantly enhanced the development of terminal inflorescences (weight and diameter) as can be seen in Table X. Analysis of variance indicated that both F values exceeded the 1% level of significance. Duncan's Multiple Range Test showed that there were significant differences among treatments. The differences in the weight and diameter of terminal inflorescences are summarized in Table X.



Figure 5. The effect of GA₃ on the diameter of terminal inflorescences of Chrysanthemum morifolium plants growing under short photoperiod.

TABLE VIII

The influence of GA₃ on the dry weight of terminal stem of Chrysanthemum morifolium (Iceberg) plants growing under short and long photoperiod regimes

A. Mean Stem Dry Weight

Treatments	Mean Stem Dry Weight in Grams	
	Short Day	Long Day
0 ppm GA ₃	1.019 ^b	1.466 ^b
5 ppm GA ₃	0.986 ^c	2.210 ^{ab}
10 ppm GA ₃	1.474 ^a	2.150 ^{ab}
50 ppm GA ₃	1.101 ^b	2.340 ^a
100 ppm GA ₃	1.160 ^b	2.394 ^a

B. Analysis of Variance

Source	DF	MS	Dry Weight Stems		
			Short Day F	MS	Long Day F
Replications	3				
Treatments	4	0.005	0.454 ^{N.S.}	0.223	7.60 ^{**}
Error	12	0.011		0.029	

N.S. Does not exceed the 5% level of significance.

**Significant at 1% level.

Numbers in each column that are not followed by the same letter are significant from each other at the 5% level of significance as judged by Duncan's New Multiple Range Test.

THESE RESULTS ARE IN ACCORD WITH THE RESULTS OF THE
 OTHER STUDIES WHICH HAVE BEEN CONDUCTED IN THE
 PAST AND WHICH HAVE SHOWN THAT THE
 EFFECT OF THE TREATMENT IS SIGNIFICANT.

TABLE I

GROUP	MEAN	STANDARD DEVIATION
CONTROL	10.0	1.0
TREATMENT	12.0	1.5
CONTROL	10.0	1.0
TREATMENT	12.0	1.5
CONTROL	10.0	1.0
TREATMENT	12.0	1.5

TABLE II

GROUP	MEAN	STANDARD DEVIATION
CONTROL	10.0	1.0
TREATMENT	12.0	1.5
CONTROL	10.0	1.0
TREATMENT	12.0	1.5
CONTROL	10.0	1.0
TREATMENT	12.0	1.5

THESE RESULTS ARE IN ACCORD WITH THE RESULTS OF THE
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TABLE IX

The influence of GA₃ on the dry weight of leaves of Chrysanthemum morifolium (Iceberg) plants growing under short and long photoperiod regimes

A. Mean Dry Weight of Leaves from Terminal Stems

Treatments	Mean Dry Weight in Grams	
	Short Day	Long Day
0 ppm GA ₃	0.481 ^c	1.060 ^a
5 ppm GA ₃	0.509 ^b	0.809 ^b
10 ppm GA ₃	0.551 ^a	1.017 ^a
50 ppm GA ₃	0.559 ^a	1.110 ^a
100 ppm GA ₃	0.557 ^a	1.090 ^a

B. Analysis of Variance

Source	DF	MS	Short Day	MS	Long Day
			F		F
Replications	3				
Treatments	4	0.0048	17.5**	0.0605	9.6**
Error	12	0.00028		0.0063	

**Significant at 1% level.

Numbers in each column that are not followed by the same letter are significant from each other at the 5% level of significance as judged by Duncan's New Multiple Range Test.

REPORT

Submitted by: [Name] Date: [Date]

Subject: [Subject]

1. Introduction

Item	Value	Unit
Item 1	100	kg
Item 2	200	kg
Item 3	300	kg
Item 4	400	kg
Item 5	500	kg
Item 6	600	kg

2. Results

Item	Value	Unit
Item 1	100	kg
Item 2	200	kg
Item 3	300	kg
Item 4	400	kg
Item 5	500	kg
Item 6	600	kg

3. Conclusion

The results of the experiment show that the [subject] is [description]. The [subject] is [description]. The [subject] is [description].

TABLE X

The influence of GA₃ on the fresh weight and diameter of terminal inflorescences of Chrysanthemum morifolium (Iceberg) plants growing under short photoperiod regime

A. Mean Weight and Diameter of Terminal Inflorescences

Treatments	Mean Weight in Gram	Mean Diameter in cm.
0 ppm GA ₃	5.022 ^b	8.34 ^b
5 ppm GA ₃	6.675 ^a	8.84 ^a
10 ppm GA ₃	6.562 ^a	8.83 ^a
50 ppm GA ₃	7.179 ^a	8.87 ^a
100 ppm GA ₃	7.412 ^a	9.20 ^a

B. Analysis of Variance

Source	DF	MS	Mean Weight F	MS	Mean Diameter F
Replications	3				
Treatments	4	3.48	5.636**	0.473	5.50**
Error	12	0.617		0.086	

**Significant at 1% level.

Numbers in each column that are not followed by the same letter are significant from each other at the 5% level of significance as judged by Duncan's New Multiple Range Test.

K. Effects of GA₃ on the Dry Weight of Terminal Inflorescences and Length of 1st Lateral Peduncles of Chrysanthemum Plants Growing under a Short Photoperiod Regime

GA₃ applications significantly increased both the dry weight of terminal inflorescences and the length of 1st lateral peduncles compared to controls. Duncan's Multiple Range Test indicated that there were significant differences among treatments both in the dry weight of terminal inflorescences and in the length of 1st lateral peduncles. The differences are summarized in Table XI.

L. Effects of GA₃ on the Chlorophyll Level in Leaves of Chrysanthemum Plants Growing Under Short and Long Photoperiod Regimes

The average chlorophyll content in leaves of chrysanthemum plants was decreased as a result of GA₃ treatment. Analysis of variance revealed that only the chlorophyll level in leaves under long day regime showed significant differences. Duncan's Multiple Range Test also indicated that there were significant differences among treatments under the long day regime only. The results are summarized in Tables XII and XIII.

DISCUSSION

The results obtained from the spraying of Chrysanthemum morifolium plants with aqueous solutions of GA₃ indicated that gibberellic acid influences the stem length of these plants grown both under long and short day regimes. However, a greater

TABLE XI

The Influence of GA₃ on dry weight of terminal inflorescences and on the length of the 1st lateral peduncle of Chrysanthemum morifolium (Iceberg) plants growing under short photoperiod regime

A. Mean Dry Weight of Terminal Inflorescences and the Length of 1st Lateral Peduncle

Treatment	Mean Dry Weight in gram	Mean Length in cm
0 ppm GA ₃	0.538 ^b	17.50 ^d
5 ppm GA ₃	0.577 ^{ab}	22.37 ^c
10 ppm GA ₃	0.581 ^{ab}	22.38 ^c
50 ppm GA ₃	0.613 ^a	29.93 ^b
100 ppm GA ₃	0.629 ^a	35.28 ^a

B. Analysis of Variance

Source	DF	MS	Dry Weight of Inflorescences F	MS	1st Lateral Peduncle F
Replications	3				
Treatments	4	0.005	3.84*	193.73	28.966**
Error	12	0.0013		6.688	

* Significant at 5% level.

**Significant at 1% level.

Numbers in each column that are not followed by the same letter are significant from each other at the 5% level of significance as judged by Duncan's New Multiple Range Test.

TABLE XII

The influence of GA₃ on the chlorophyll levels in Chrysanthemum morifolium (Iceberg) plants growing in short photoperiod regime

A. Absorbancy Readings of Chlorophyll Extracts from Leaves

Treatments	645 mu	663 mu
0 ppm GA ₃	0.223	0.556
5 ppm GA ₃	0.198	0.495
10 ppm GA ₃	0.189	0.470
50 ppm GA ₃	0.140	0.365
100 ppm GA ₃	0.176	0.485

B. Absorbancy Readings Converted to mg Chlorophyll per mg Fresh Weight Basis

Treatments	Chlorophyll a	Chlorophyll b
0 ppm GA ₃	0.735	0.242
5 ppm GA ₃	0.654	0.214
10 ppm GA ₃	0.621	0.204
50 ppm GA ₃	0.483	0.145
100 ppm GA ₃	0.475	0.176

C. Total Chlorophyll (a + b) in mg per mg Fresh Weight of Leaves

Treatments	Chlorophyll (a + b)
0 ppm GA ₃	0.977 ^a
5 ppm GA ₃	0.868 ^a
10 ppm GA ₃	0.825 ^a
50 ppm GA ₃	0.628 ^a
100 ppm GA ₃	0.651 ^a

D. Analysis of Variance on the Level of Total Chlorophyll Content

Source of variation	SS	DF	MS	F
Replications	0.044	1		
Treatments	0.189	4	0.0472	1.305 ^{N.S.}
Error	0.145	4	0.0362	

N.S. Does not exceed the 5% level of significance.

Numbers in column that are not followed by the same letter are significant from each other at the 5% level of significance as judged by Duncan's New Multiple Range Test.

TABLE XIII

The influence of GA₃ on chlorophyll levels in leaves of Chrysanthemum morifolium (Iceberg) plants growing under long photoperiod regime

A. Absorbancy Readings of Chlorophyll Extracts from Leaves

Treatments	645 mu	663 mu
0 ppm GA ₃	0.458	1.177
5 ppm GA ₃	0.253	0.748
10 ppm GA ₃	0.268	0.760
50 ppm GA ₃	0.185	0.514
100 ppm GA ₃	0.225	0.655

B. Absorbancy Readings Converted to mg Chlorophyll/mg Fresh Weight Basis

Treatments	Chlorophyll a	Chlorophyll b
0 ppm GA ₃	1.557	0.482
5 ppm GA ₃	0.785	0.189
10 ppm GA ₃	1.008	0.253
50 ppm GA ₃	0.526	0.179
100 ppm GA ₃	0.869	0.123

C. Total Chlorophyll (a + b) in mg/mg Fresh Weight of Leaves

Treatments	Chlorophyll (a + b)
0 ppm GA ₃	2.039a
5 ppm GA ₃	0.971 b
10 ppm GA ₃	1.261 b
50 ppm GA ₃	0.705 c
100 ppm GA ₃	0.992 b

D. Analysis of Variance on the Level of Total Chlorophyll Content

Source of Variation	SS	DF	MS	F
Replications	0.358	1		
Treatments	1.947	4	0.487	16.07**
Error	0.121	4	0.0303	

** Significant at 1% level.

Numbers in column that are not followed by the same letter are significant from each other at the 5% level of significance as judged by Duncan's New Multiple Range Test.

Table 1

Table 1 shows the results of the regression analysis for the dependent variable Y against the independent variables X_1, X_2, X_3, X_4, X_5 . The results are presented in the following table.

Table 1. Results of the regression analysis for the dependent variable Y against the independent variables X_1, X_2, X_3, X_4, X_5 .

Variable	Parameter	Standard Error
Intercept	1.234	0.123
X_1	0.456	0.056
X_2	-0.789	0.089
X_3	0.123	0.034
X_4	0.567	0.067
X_5	-0.234	0.045

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increase in stem length occurred in treated plants under short photoperiod than under long photoperiod. A possible explanation for this differential in response may be found in the hypothesis of Stuart and Cathey (57) who suggest that gibberellin induces maximum response when the growth conditions are adversely influenced by temperature, nutrition, short photoperiod or other environmental factors. Possibly under these conditions synthesis of both native gibberellin and inhibitor is retarded, permitting the greater response from exogenous gibberellin. It has also been reported by Wittwer and Bukovac (58) that crop response is often greatest when growth is retarded by subnormal temperature, low light intensities, short days and/or deficient moisture.

The stem strength of the plants growing under both long and short photoperiod decreased with GA₃ treatments. The decrease in the stem strength was influenced by two factors, photoperiod and GA₃ treatment. The stem strength of control plants (without GA₃ treatment) in long and short photoperiod regimes was different. Possible explanation as to why the stems were weaker under short photoperiod conditions are: 1) The chrysanthemum being a short day response plant, carbohydrates are used for the flower bud initiation and development during the short photoperiod regime. No flower buds initiated and developed under the long day regime and more photosynthate would be available for vegetative growth resulting in stronger stems. 2) During the short photoperiod regime lateral peduncles developed and elongated. No lateral stems developed and elongated under the long

day regime. Hence more carbohydrate would be available for thicker cell wall development in the main stems of plants under the long day regime than for those under the short day regime. The other factor which decreased the stem strength was treatment with GA_3 . Possible explanations as to why the stems were weaker with GA_3 treatments are: 1) The plants which were treated with GA_3 increased in auxin content (48). Auxin tends to soften cell walls and increase their ability to absorb water, thus keeping them in a plastic condition which would result in weakening the stem strength. 2) Plants which are treated with GA_3 have increased levels of reducing sugar and lower levels of starch content (27). Hence the plant composition may be altered from a relatively high polysaccharide content, in the form of secondary wall thickening giving added stem strength, to monosaccharides which may be used for the rapid growth of leaves.

The fresh weight of tops including stem and leaves both in short and long day lengths was increased with GA_3 treatment. These results agree with those of Hayashi (28) who suggests that the photosynthetic activity per unit leaf area does not change as a result of gibberellic acid treatment, but owing to the increase in leaf area, the photosynthetic activity of the whole plant increases. Such over-all increase in photosynthesis results in increased weight. It also has been reported by Brian (9) that the increased weight is believed to be a secondary effect of increased leaf growth.

The top fresh weight was affected by photoperiod as well as by GA₃ treatment. When we compare control plants (without GA₃ treatment) grown under short and long day regimes, we find that the short photoperiod was effective in stimulating increased top fresh weight (mainly stem fresh weight). Under short photoperiod many lateral peduncles developed and elongated, but there were no lateral stems formed under long photoperiod conditions. Therefore, the added fresh weight under the short day regime was due to lateral branching.

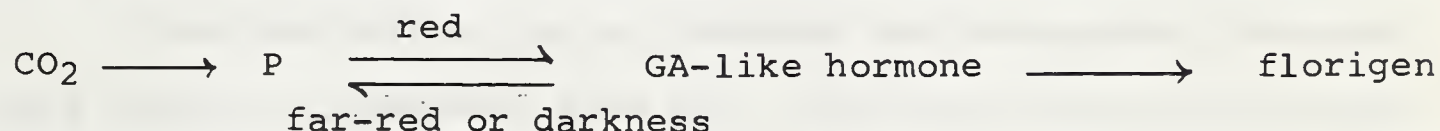
The number of internodes formed both in short and long day regimes was increased with GA₃ treatment. The number of internodes of terminal stem increase was mainly due to the stem elongation. Elongation results primarily from an increase in the length of internodes (15) but sometimes from an increase in the number of nodes (11, 22). Since Chrysanthemum morifolium is a short day plant, it continues vegetative growth under long photoperiod. Therefore, a greater response to GA₃ could be expected with respect to increase in the number of internodes under long photoperiod than under short photoperiod conditions. As shown in Table IV the control plants had an average of 18.25 internodes but the plants which received the 100 ppm treatment had up to 24.50 internodes under long day conditions. Since treated plants under long photoperiod did not flower we can conclude that the application of GA₃ does not replace the effect of short photoperiod. In view of the fact that gibberellic acid can replace vernalization and long day requirement (6, 34), one might expect it to induce flowering in short day plants under long day

The following is a list of the names of the persons who have been elected to the office of Justice of the Peace for the year 1900. The names are given in alphabetical order of their surnames. The names of the persons who have been elected to the office of Justice of the Peace for the year 1900 are: [illegible names]

JUSTICES OF THE PEACE

The following is a list of the names of the persons who have been elected to the office of Justice of the Peace for the year 1900. The names are given in alphabetical order of their surnames. The names of the persons who have been elected to the office of Justice of the Peace for the year 1900 are: [illegible names]

conditions. However, Brian (9) offers an explanation for this apparent discrepancy. He suggests that in plants sensitive to photoperiod, a GA-like hormone is produced in the leaves during the daily light period. Flowering responses can then be explained if it is assumed 1) that long day plants are conditioned to elaborate florigen (flowering hormone) only when levels of the GA-like hormone in the leaves are maintained at a high level for a critical period, and 2) that short day plants are conditioned to produce florigen only when the levels of GA-like hormone in the leaves are maintained at a lower level than for long day plants for the critical period. Once florigen is formed, initiation of flower primordia follows. The complete scheme may be represented thus:



1. P = inactive precursor of GA-like hormone.
2. The concentration of GA-like hormone will tend to be higher in plants exposed to long photoperiods than in plants exposed to short photoperiod. Red light exposure during the dark period will increase the hormone concentration and treatment with far-red will reduce its concentration.
3. The florigen converted from the GA-like hormone will be controlled by the concentration of GA-like hormone differentially in long day plants and short day plants.
4. Florigen is the name applied to a proposed flower inducing hormone the existence of which has never been confirmed. The term was first used by Chailakhyan in Russia in 1936.

In long day plants grown under long day conditions, vegetative shooting and flowering are almost invariably associated. This is consistent with the scheme outlined above since vigorous vegetative growth would be expected if endogenous levels of GA-like hormones were high.

In short day plants, flowering and shoot extension are naturally dissociated, flowering taking place only under short day conditions, whereas maximum vegetative growth is obtained only under long-day conditions. This is accounted for in the scheme by the supposition that in short day plants florigen synthesis is inhibited at the high levels of GA-like hormone which would accumulate in long days, when vegetative growth would be favored. This view is consistent with the effects of exogenous GA.

The size and weight of terminal inflorescences increased as a result of treatment with GA₃. This was due to an increase in floret size as has been reported by others (14, 15, 20, 37, 56). Both increased cell division and cell elongation account for this increase in floret size (53).

The root growth was slightly decreased by treatment with GA₃. Although the methods of application differed in these experiments the results agree with those of Marth, et. al. (40) on soy bean plants and Alvim (1) on bean plants. It is suggested by Brian and Hemming (6) that such a response is due to the redistribution of materials within the plants from root to shoot.

The dry weight of leaves and stems, generally speaking, was increased with GA₃ treatment. It has been reported by

Brian (9) that under some circumstances, with some plant species, treatment with GA_3 results in increased dry weight. This is mainly due to carbon fixation and is believed to be a secondary effect of increased leaf growth. The fact that the author found only small increases in dry weight would tend to support Brian's suggestion.

The dry weight of stems both under long and short day regimes was increased with GA_3 treatment. Alvim (1) suggests the increase in net assimilation rate (photosynthesis) caused by GA_3 results from a more rapid translocation of photosynthate from the leaves to stems. When we compare plants in long day and short day regimes, it indicates a more rapid translocation under the long day regime on the basis of Alvim's hypothesis.

The chlorophyll level of the plants decreased both under long and short photoperiod as a result of GA_3 treatment. Although the method of application differed these results agree with those of Brian and Hemming (7) who reported that increased plant growth stimulated by gibberellic acid is generally accompanied by a paling of the leaves. Brian (9) also reported that the leaves formed after treatment are noticeably paler in color than those formed earlier. Wittwer and Bukovac (58) also reported that new growth after GA_3 treatment is often paler green or chlorotic. This is a general response to gibberellin. Wolf and Haber (60) reported that the paling in very young seedlings resulted solely from a failure of chlorophyll synthesis to keep pace with increased cell expansion. Besides this simple dilution of chlorophyll, the apparent chlorosis in older plants

must also result from the delayed effect of malnutrition, not to direct action of the gibberellin on formation or destruction of chlorophyll. When we compare the chlorophyll content in control plants in both long and short day regimes, the plants under long photoperiod have a higher chlorophyll level. A possible explanation may be that the rate of photosynthetic activity is proportional to the chlorophyll concentration and the photosynthetic activity in turn depends upon the light intensity and photoperiod (4).

IV. THE EFFECTS OF GA₃ ON GROWTH AND FLOWERING OF Antirrhinum
majus PLANTS GROWING IN CARBON DIOXIDE ENRICHED AND NATURAL
ATMOSPHERE

The majority of the experiments relating to the effects of GA₃ on plants have been done in natural atmosphere. Reports on the effects of GA₃ on plants growing in carbon dioxide enriched atmosphere in greenhouses are few.

In order to elucidate the effect of GA₃ on snapdragon plants in carbon dioxide enriched conditions, experiments were conducted in the greenhouses during the winter months of 1964 - 1965.

MATERIALS AND METHODS

The snapdragon (variety Utah White) was selected as the test plant material because of its short life cycle. General commercial methods outlined by Laurie and Kiplinger (36) were followed except where specific treatments were involved.

The seeds were sowed in a steam-sterilized soil mixture (3 soil, 2 sand and 1 peat) in a flat on December 2, 1964. On December 28, 1964, all the seedlings were benched at a spacing 4 x 6 inches. Half the seedlings were benched in a greenhouse compartment with natural atmosphere (approximately 300 ppm of CO₂) and the remainder in a compartment with CO₂ enriched atmosphere (up to 1000 ppm CO₂ during the hours of daylight).

The experimental design was a randomized block design with four treatments replicated three times. Each treatment

contained 6 plants. The outside rows of the plants along the edges of the benches served as guard rows.

The concentrations of GA₃ used were as follows:

<u>Treatment</u>	<u>Concentration</u>
1	0 ppm
2	10 ppm
3	50 ppm
5	100 ppm

As soon as the plants were established, they were treated with GA₃ as a foliar spray. The apical region and the expanding leaves of the plants were sprayed with a hand atomizer until the excess solution began to run off. Approximately 0.5 ml of solution was required per plant. Twice weekly applications for a total of 6 applications were made.

A. General Measurement Determination

The plants were harvested during the week of April 19 to 25, 1965. Measurements were taken of the stem length (from the tip of the inflorescence to the bottom of the stem), inflorescence length, fresh weight of tops and root length.

B. Chlorophyll Determination

Chlorophyll determinations were the same as stated previously except that the leaf samples used in this experiment were 50 mg of dry leaves from each group of treated plants both in carbon dioxide enriched and natural atmospheres.

RESULTS

1. Effects of GA₃ on the Length of Inflorescences of Snapdragon Plants Growing in CO₂ Enriched and Natural Atmospheres

There was a trend toward an increase in the average length of inflorescences of snapdragon plants growing in CO₂ enriched and natural atmospheres as a result of GA₃ treatment. However, Duncan's Multiple Range Test indicated that there were significant differences among treatments only in CO₂ enriched atmosphere. The differences in length of inflorescences are summarized in Table XIV.

2. Effects of GA₃ on the Stem Length of Snapdragon Plants Growing in CO₂ Enriched and Natural Atmospheres

GA₃ applications significantly increased the stem length of snapdragon plants growing in CO₂ enriched and natural atmospheres compared to control plants. Analysis of variance revealed that both of the F values exceeded the 1% level of significance. Duncan's Multiple Range Test showed that there were significant differences among treatments. The differences in stem length are summarized in Table XV.

3. Effects of GA₃ on Fresh Weight of Tops of Snapdragon Plants Growing in CO₂ Enriched and Natural Atmospheres

There was a trend toward an increase in the average top fresh weight of snapdragon plants growing in CO₂ enriched and natural atmospheres as a result of GA₃ treatment. Duncan's Multiple Range Test, however, revealed that there were significant

The first part of the paper is devoted to a general
discussion of the problem of the existence of
solutions of the system of equations (1) and (2).
In the second part we shall consider the case of
a linear system of equations. In the third part
we shall consider the case of a nonlinear system
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the case of a system of equations with variable
coefficients.

TABLE XIV

The influence of GA₃ on the length of inflorescences of Antirrhinum majus (Utah White) plants growing in CO₂ enriched and natural atmospheres

A. Mean Inflorescence Length

Treatments	Mean inflorescence length in cm	
	CO ₂ Enriched Atmosphere	Natural Atmosphere
0 ppm GA ₃	18.33 ^b	16.54 ^a
10 ppm GA ₃	18.85 ^a	16.63 ^a
50 ppm GA ₃	19.96 ^a	18.01 ^a
100 ppm GA ₃	20.15 ^a	18.43 ^a

B. Analysis of Variance

Source	DF	MS	Mean inflorescence length		
			CO ₂ Enriched Atmosphere F	MS	Natural Atmosphere F
Replications	2				
Treatments	3	2.712	22.048**	2.787	2.281 ^{N.S.}
Error	6	0.123		1.222	

N.S. Does not exceed the 5% level of significance.

** Significant at 1% level.

Numbers in each column that are not followed by the same letter are significant from each other at the 5% level of significance as judged by Duncan's New Multiple Range Test.

Figure 6. The effect of GA_3 on the length of inflorescences of Antirrhinum majus (Utah White) growing in CO_2 enriched and natural atmosphere.

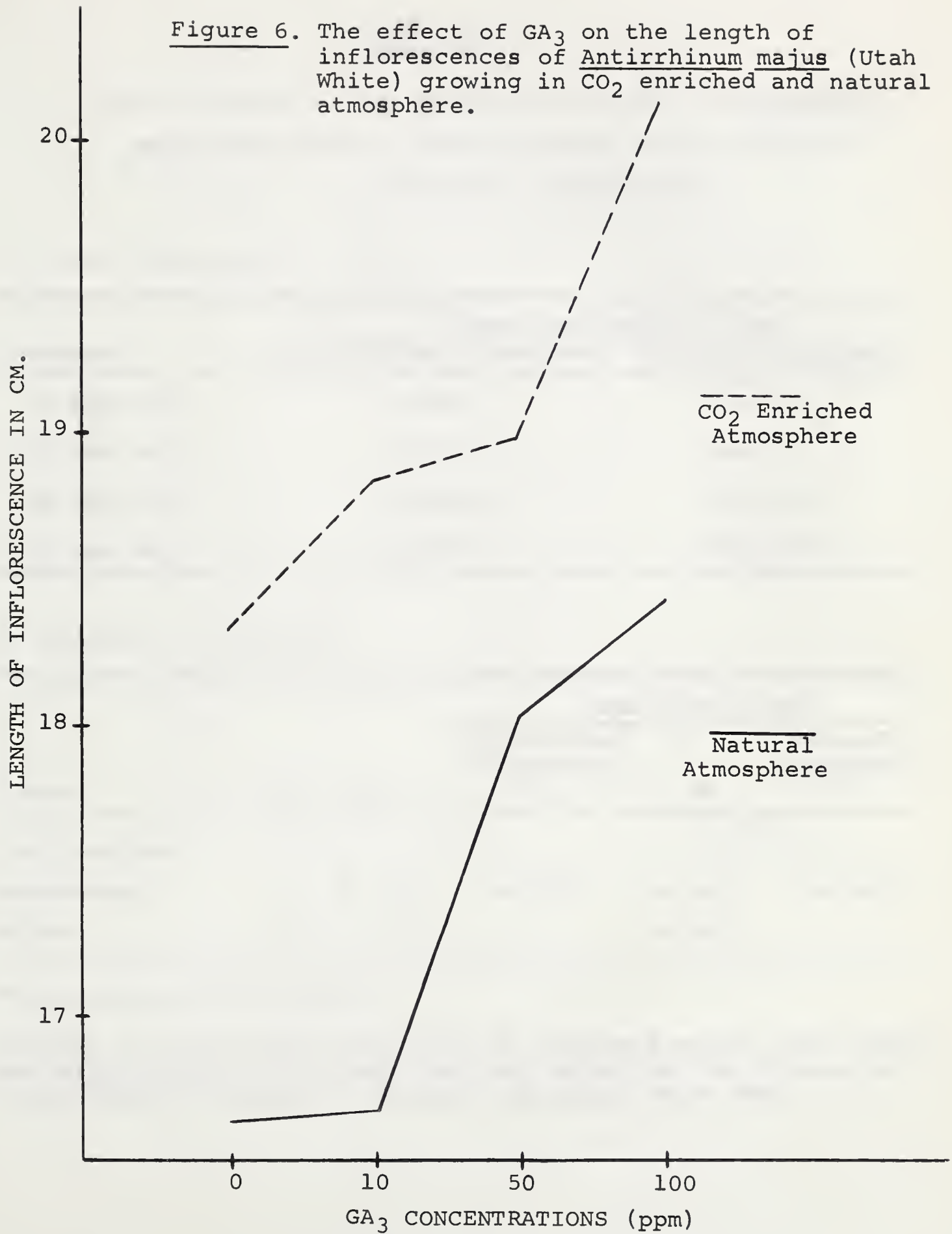




TABLE XV

The influence of GA₃ on the stem length of Antirrhinum majus (Utah White) plants growing in CO₂ enriched and natural atmospheres

A. Mean Stem Length

Treatment	Mean Stem Length in cm	
	CO ₂ Enriched Atmosphere	Natural Atmosphere
0 ppm GA ₃	91.443 ^c	79.700 ^d
10 ppm GA ₃	94.410 ^b	84.577 ^c
50 ppm GA ₃	96.780 ^a	86.630 ^b
100 ppm GA ₃	98.216 ^a	88.656 ^a

B. Analysis of Variance

Source	DF	MS	Mean Stem Length		
			CO ₂ Enriched Atmosphere F	MS	Natural Atmosphere F
Replications	2				
Treatments	3	26.332	46.61**	44.249	68.603**
Error	6	0.565		0.645	

**Significant at 1% level.

Numbers in each column which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's Multiple Range Test.

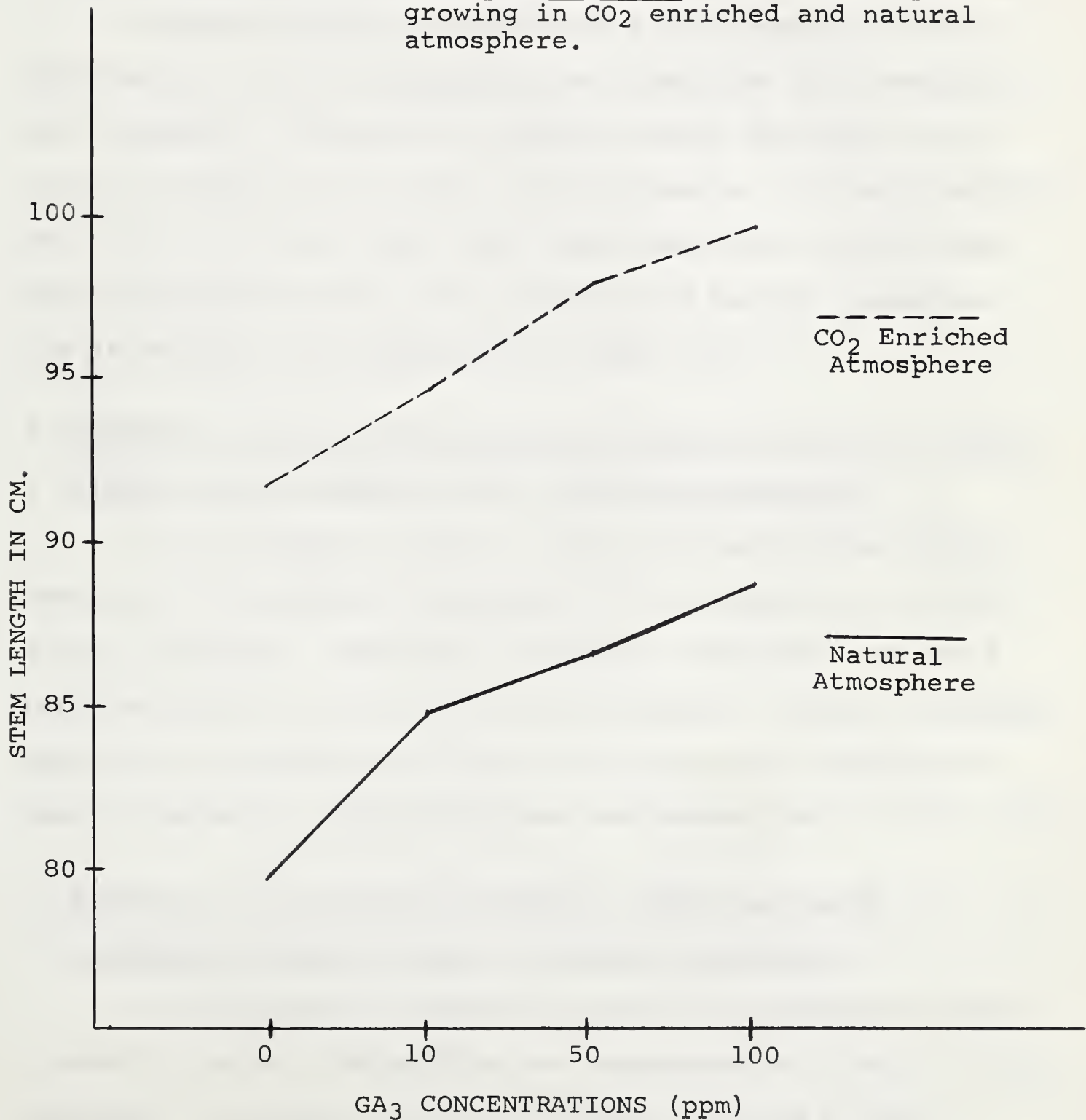
The following is a list of the names of the persons who have been
 appointed to the various offices of the County of ...
 for the year 1900.

County of ...			Office		Name	
County	Office	Name	County	Office	Name	County
...
...
...
...

County of ...			Office		Name	
County	Office	Name	County	Office	Name	County
...
...
...
...

The following is a list of the names of the persons who have been
 appointed to the various offices of the County of ...
 for the year 1900.

Figure 7. The effect of GA₃ on the stem length of Antirrhinum majus (Utah White) plants growing in CO₂ enriched and natural atmosphere.





differences between treatments and the control only in CO₂ enriched atmosphere. The differences in fresh weight of tops are summarized in Table XVI.

4. Effects of GA₃ on the Root Length of Snapdragon Plants Growing in CO₂ Enriched and Natural Atmospheres

The root length of snapdragon plants growing in CO₂ enriched and natural atmospheres was decreased as a result of GA₃ treatment. Analysis of variance showed that both the F values exceeded the 1% level of significance. Duncan's Multiple Range Test indicated that there were significant differences among treatments both in CO₂ enriched and natural atmosphere. The differences are summarized in Table XVII.

5. Effects of GA₃ on the Chlorophyll Levels in Leaves of Snapdragon Plants Growing in CO₂ enriched Atmosphere

The chlorophyll levels in leaves of snapdragon plants growing in CO₂ enriched atmosphere were decreased as a result of GA₃ treatment. Analysis of variance indicated that the F value exceeded the 1% level of significance. Duncan's Multiple Range Test revealed that there were significant differences among treatments. The differences are summarized in Table XVIII.

6. Effects of GA₃ on the Chlorophyll Levels in Leaves of Snapdragon Plants Growing in Natural Atmosphere

The chlorophyll content in leaves of snapdragon plants growing in natural atmosphere were decreased as a result of GA₃ treatment. Analysis of variance showed that the F value

TABLE XVI

The influence of GA₃ on the fresh weight of tops of Antirrhinum majus (Utah White) plants growing in CO₂ enriched and natural atmosphere

A. Mean Fresh Weight

Treatments	Mean Fresh Weight in gms	
	CO ₂ Enriched Atmosphere	Natural Atmosphere
0 ppm GA ₃	71.266 ^b	54.296 ^a
10 ppm GA ₃	75.426 ^a	59.446 ^a
50 ppm GA ₃	78.010 ^a	59.356 ^a
100 ppm GA ₃	78.450 ^a	68.353 ^a

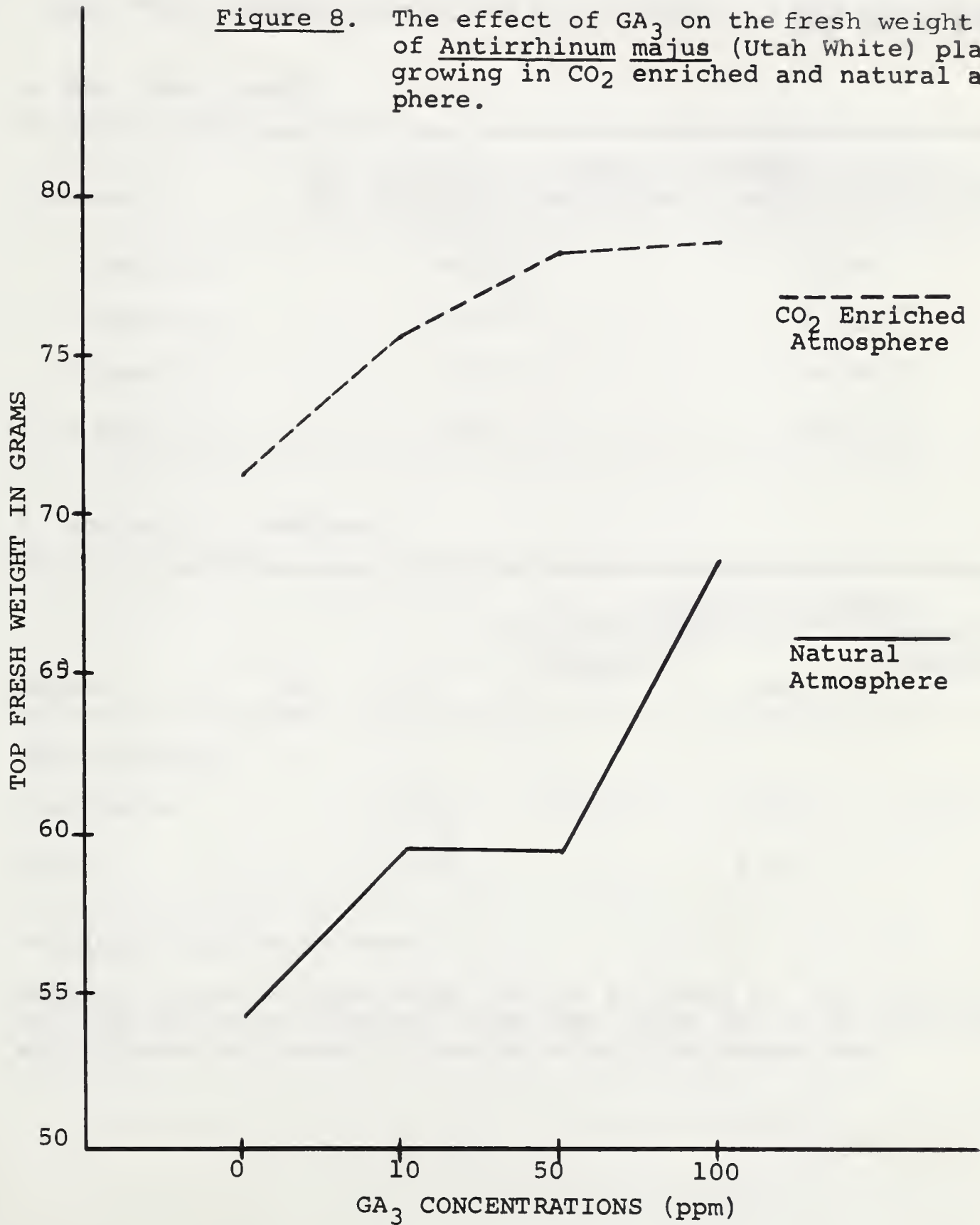
B. Analysis of Variance

Source	DF	MS	Mean Fresh Weight		
			CO ₂ Enriched Atmosphere	MS	Natural Atmosphere
			F		F
Replications	2				
Treatments	3	17.439	1.843 ^{N.S.}	102.498	1.833 ^{N.S.}
Error	6	9.46		54.407	

N.S. Does not exceed the 5% level of significance.

Numbers in each column which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's Multiple Range Test.

Figure 8. The effect of GA_3 on the fresh weight of tops of Antirrhinum majus (Utah White) plants growing in CO_2 enriched and natural atmosphere.



1. The following is a graph showing the variation of the rate of reaction with temperature. The graph shows that the rate of reaction increases with temperature up to a certain point and then decreases.

Figure 1

Rate of reaction

Temperature



TABLE XVII

The influence of GA₃ on the root length of Antirrhinum majus (Utah White) plants growing in CO₂ enriched and natural atmosphere

A. Mean Root Length

Treatments	Mean Root Length in cm	
	CO ₂ Enriched Atmosphere	Natural Atmosphere
0 ppm GA ₃	18.10 ^a	16.49 ^a
10 ppm GA ₃	15.95 ^b	14.20 ^b
50 ppm GA ₃	14.33 ^c	14.36 ^b
100 ppm GA ₃	13.19 ^c	14.13 ^b

B. Analysis of Variance

Source	DF	MS	Mean Root Length	
			CO ₂ Enriched Atmosphere F	Natural Atmosphere F
Replications	2			
Treatments	3	13.603	27.724**	3.853
Error	6	0.509		0.303

** Significant at 1% level.

Numbers in each column which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's Multiple Range Test.

The following is a list of the names of the persons who have been
 admitted to the office of the Secretary of the Board of Education
 since the last meeting of the Board.

In January, 1900		
Name	Age	Address
John A. Smith	25	123 Main St.
John B. Smith	25	123 Main St.
John C. Smith	25	123 Main St.
John D. Smith	25	123 Main St.

In February, 1900		
Name	Age	Address
John E. Smith	25	123 Main St.
John F. Smith	25	123 Main St.
John G. Smith	25	123 Main St.
John H. Smith	25	123 Main St.

The following is a list of the names of the persons who have been
 admitted to the office of the Secretary of the Board of Education
 since the last meeting of the Board.

Figure 9. The effects of GA_3 on the root length of Antirrhinum majus (Utah White) plants growing in CO_2 enriched and natural atmospheres.

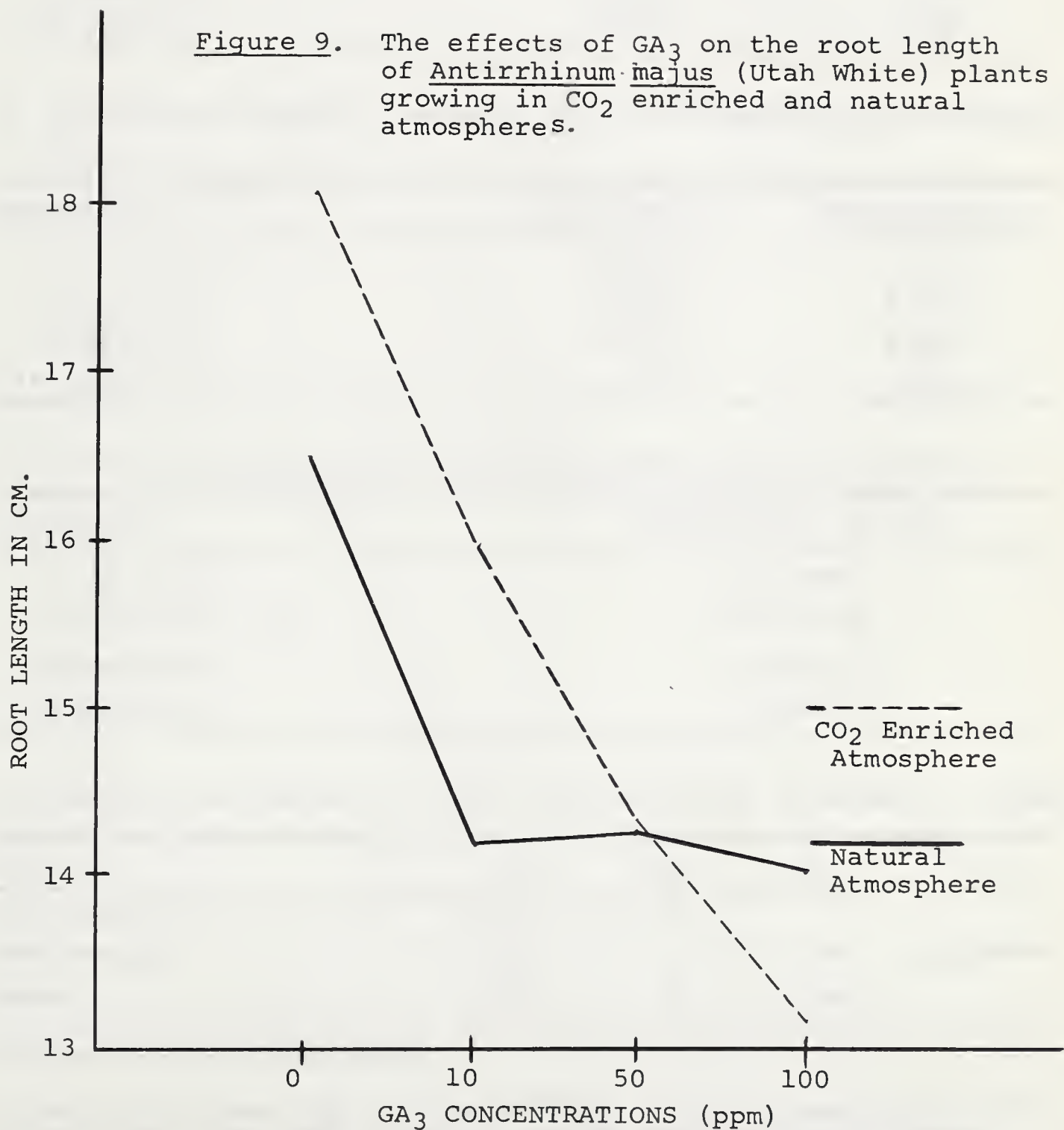


TABLE XVIII

The influence of GA₃ on the chlorophyll level in Antirrhinum majus (Utah White) plants growing in CO₂ enriched atmosphere

A. Absorbancy Readings of Chlorophyll Extracts from Leaves

Treatments	645 mu	663 mu
0 ppm GA ₃	0.275	0.812
10 ppm GA ₃	0.185	0.533
50 ppm GA ₃	0.171	0.486
100 ppm GA ₃	0.162	0.460

B. Absorbancy Readings Converted to mg Chlorophyll/mg Dry Weight Basis

Treatments	Chlorophyll a	Chlorophyll b
0 ppm GA ₃	12.930	2.978
10 ppm GA ₃	8.091	2.176
50 ppm GA ₃	6.110	2.042
100 ppm GA ₃	5.713	1.814

C. Total Chlorophyll (a + b) in mg/mg Dry Weight of Leaves

Treatments	Chlorophyll (a + b)
0 ppm GA ₃	15.908 ^a
10 ppm GA ₃	11.267 ^b
50 ppm GA ₃	8.152 ^c
100 ppm GA ₃	7.527 ^c

D. Analysis of Variance on the Level of Total Chlorophyll Content

Source	SS	DF	MS	F
Replications	0.961	2		
Treatments	107.831	3	35.943	55.895**
Error	3.855	6	0.643	

**Significant at 1% level.

Numbers in column that are not followed by the same letter are significant from each other at the 5% level of significance as judged by Duncan's New Multiple Range Test.

exceeded the 1% level of significance. Duncan's Multiple Range Test indicated that there were significant differences among treatments. The differences are summarized in Table XIX.

DISCUSSION

The control plants in CO₂ enriched atmosphere showed a remarkable increase in the length of inflorescences, the length of stems, the top fresh weight, the root length and chlorophyll (a + b) content when compared with control plants in natural atmosphere. The significant differences can be attributed to the CO₂, an important raw material for photosynthesis. Under normal conditions, plant growth depends upon the photosynthetic activity and photosynthesis, in turn, is dependent upon the concentrations of CO₂ and light intensity.

Both the length of inflorescences and the stem length were increased as a result of GA₃ treatment both in CO₂ enriched and natural atmosphere; but there was a higher percentage of increase of stem length due to GA₃ treatment in natural atmosphere. A possible explanation may be found in the hypothesis of Stuart and Cathey (57) who suggest that the growth of a plant is a net result of the influence of growth promoting materials versus growth inhibiting materials. It has been observed that GA often induces maximum responses when growth is adversely influenced by temperature, nutrition or other environmental factors. Possibly under these conditions synthesis of both native gibberellin and inhibitors is retarded, permitting the greater response from exogenous gibberellin. Wittwer and

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Information

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TABLE XIX

The influence of GA₃ on the chlorophyll level in Antirrhinum majus (Utah White) plants growing in natural atmosphere

A. Absorbancy Readings of Chlorophyll Extracts from Leaves

Treatments	645 mu	663 mu
0 ppm GA ₃	0.245	0.715
10 ppm GA ₃	0.215	0.598
50 ppm GA ₃	0.182	0.480
100 ppm GA ₃	0.174	0.445

B. Absorbancy Readings Converted to mg Chlorophyll/mg Dry Weight Basis

Treatments	Chlorophyll a	Chlorophyll b
0 ppm GA ₃	5.691	2.748
10 ppm GA ₃	4.754	1.252
50 ppm GA ₃	3.811	1.118
100 ppm GA ₃	3.529	1.106

C. Total Chlorophyll (a + b) in mg/mg Dry Weight of Leaves

Treatments	Chlorophyll (a + b)
0 ppm GA ₃	8.439 ^a
10 ppm GA ₃	6.006 ^b
50 ppm GA ₃	4.929 ^b
100 ppm GA ₃	4.635 ^b

D. Analysis of Variance on the Level of Total Chlorophyll Content

Source	SS	DF	MS	F
Replications	0.048	2		
Treatments	29.692	3	9.897	15.156**
Error	3.92	6	0.653	

**Significant at 1% level.

Numbers in column that are not followed by the same letter are significant from each other at the 5% level of significance as judged by Duncan's New Multiple Range Test.

Table 1

Summary of the results of the analysis of variance for the different treatments and the different periods of observation.

Treatment	Period	Mean	Standard Error
1	1	10.5	0.5
2	1	11.2	0.5
3	1	10.8	0.5
4	1	11.0	0.5

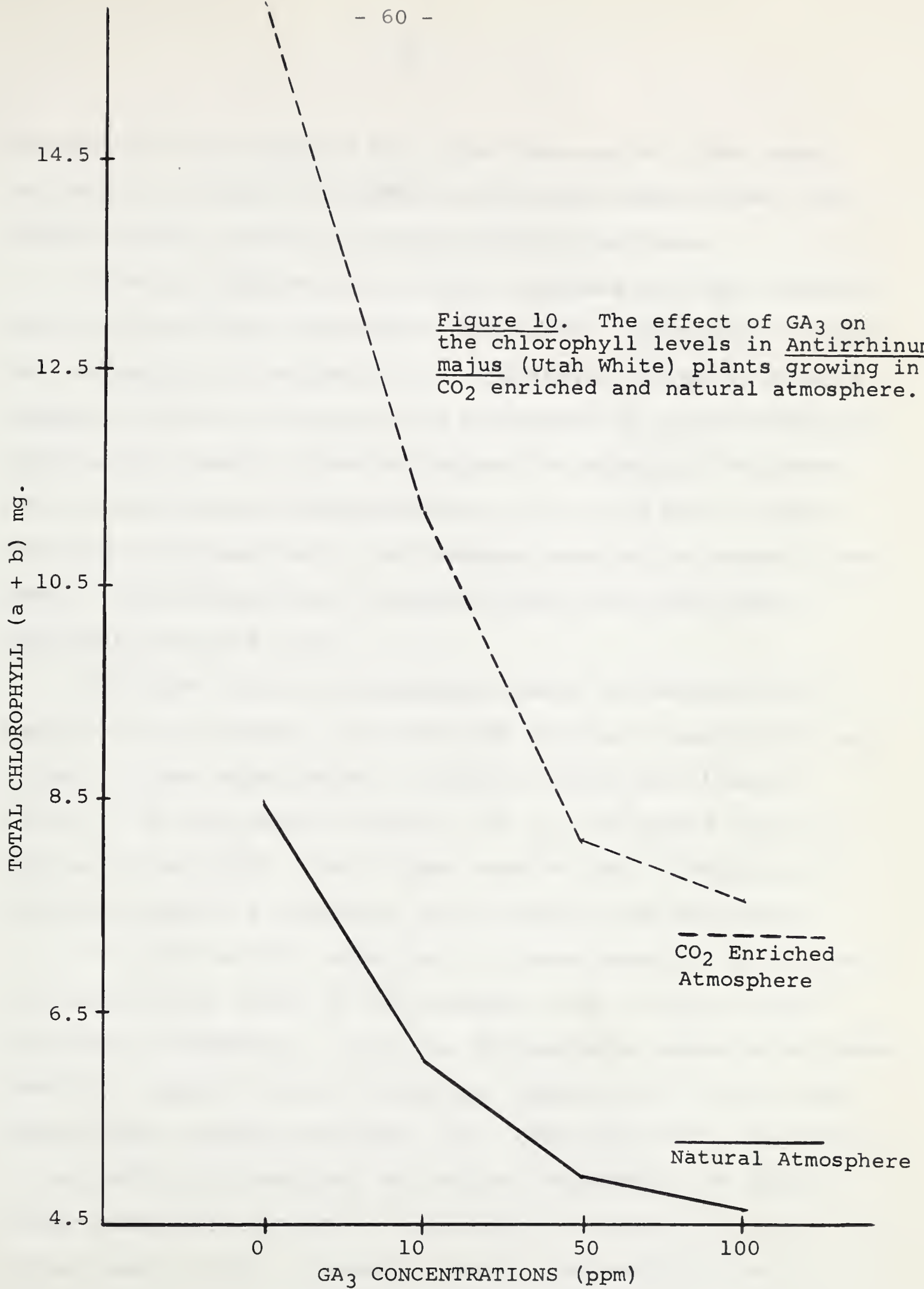
Treatment	Period	Mean	Standard Error
1	2	12.0	0.5
2	2	12.5	0.5
3	2	12.2	0.5
4	2	12.4	0.5

Treatment	Period	Mean	Standard Error
1	3	13.0	0.5
2	3	13.5	0.5
3	3	13.2	0.5
4	3	13.4	0.5

Treatment	Period	Mean	Standard Error
1	4	14.0	0.5
2	4	14.5	0.5
3	4	14.2	0.5
4	4	14.4	0.5

The results of the analysis of variance show that there are significant differences between the different treatments and the different periods of observation.

Figure 10. The effect of GA₃ on the chlorophyll levels in Antirrhinum majus (Utah White) plants growing in CO₂ enriched and natural atmosphere.



The following curves are plotted for the
 reaction of the acid with the base
 at various temperatures. The curves
 show that the reaction is exothermic.



Bukovac (53) also reported that plant response is often greatest when the growth is retarded by subnormal temperatures, low light intensity, short day and/or deficient moisture.

The top fresh weight was also increased with GA₃ treatment both in CO₂ enriched and natural atmosphere. Again these results are in keeping with Hayashi's (28) hypothesis as well as Brian's suggestion (9) as outlined in the discussion of the chrysanthemum experimental results. When we compare the effects of GA₃ under CO₂ enriched and natural atmosphere, it is found that a higher percentage increase due to GA treatment occurred in natural atmosphere. These results are in keeping with Stuart and Cathey's hypothesis outlined above.

The root length of snapdragon plants was decreased as a result of GA₃ treatment. Although the methods of application may differ in these experiments, the results agree with those of Alvim (1) on bean plants, Hayashi (28) on rice plants and Leak (35) on yellow birch. Again these results are in keeping with Brian and Hemming's hypothesis as outlined in the discussion.

The chlorophyll content in the leaves also was influenced to a significant degree by GA₃ treatment both in CO₂ enriched and natural atmosphere. As in the chrysanthemum experiments, these results support those of Brian and Hemming (7), Wittwer and Bukovac (58) and Wolf and Haber (60). When we compare the effect of GA₃ both in CO₂ enriched and natural atmosphere, we find a higher percentage increase in chlorophyll content under the higher level of CO₂. A possible explanation may be found in the work of Bonner and Galston (4) who reported that the

chlorophyll content of the cell is perhaps more closely associated with photosynthesis performance than is any other internal factor. Photosynthetic rate is dependent upon the CO₂ concentration and light intensity. As mentioned previously, the author's results support the hypothesis of Hayashi (28) and Brian (9) that one of the major effects of gibberellin is to increase the photosynthetic activity. Conlombe and Daquin (18) also reported that gibberellin increases photosynthesis in tomato plants. Therefore, we may conclude that under normal conditions, both CO₂ enriched atmosphere and gibberellic acid increase the photosynthetic activity and consequently increase the chlorophyll content.

V. THE EFFECTS OF GA₃ ON GROWTH AND FLOWERING OF Dianthus caryophyllus PLANTS GROWING IN CARBON DIOXIDE ENRICHED AND NATURAL ATMOSPHERES

The purpose of the experiment was to determine the effects of GA₃ on the diameter of terminal flowers, stem length, stem and terminal flower weight and terminal stem strength of Dianthus caryophyllus plants growing in CO₂ enriched (winter months only) and natural atmospheres.

MATERIALS AND METHODS

Experimental work was conducted in the greenhouses. General cultural methods as outlined by Laurie and Kiplinger (31 b) were followed except where specific treatments were involved.

Rooted cuttings of the variety William Sim were used in this experiment. On December 14, 1964, all the cuttings were benched at a spacing of 8 x 4 inches. Half the seedlings were benched in a greenhouse compartment with natural atmosphere (approximately 300 ppm of CO₂) and the remainder in a compartment with CO₂ enriched atmosphere (up to 1000 ppm CO₂ during the hours of daylight during the winter months). All plants were given a single pinch during the period January 8 to March 10, 1965. As soon as the flower buds just began to show color, the plants were treated with GA₃ as a soil drench. Drench applications were used because preliminary experiments indicated that spray applications with aqueous mixtures of GA₃ had no effect on carnation plants. The GA₃ concentrations

were as follows:

<u>Treatments</u>	<u>Concentrations</u>
1	0 ppm
2	50 ppm
3	100 ppm
4	500 ppm
5	1000 ppm

The experimental design used in this experiment was a randomized block design with 5 treatments replicated four times. Each treatment plot contained 6 plants. The outside rows of plants along the edges of the benches served as guard rows.

The CO₂ concentrations used were up to 1000 ppm CO₂ during the hours of daylight (November 1 to April 15). After April 15, 1965, ventilation of the greenhouse was required regularly and high levels of CO₂ could no longer be maintained. Although automatic temperature controls were used it was difficult to maintain strict day temperature control in both compartments. The compartment to which CO₂ was added would tend to be 5° to 15° F warmer than the compartment with normal atmosphere during sunny days.

1. General Measurement Determination

The terminal flowers including the flower stem to the fifth node below the flower, were harvested during the week of August 1 to August 6, 1965. Measurements were taken of the length (from base of flower to fifth node below), the fresh weight and the diameter of the terminal flowers.

Continued from

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2. Stem Strength Determination

Stem strength determinations were the same as stated previously except the upper 40 cm of the terminal shoot of each test plant were removed for stem strength determination.

RESULTS

Five days after the first spray application of aqueous solutions of GA₃ on the plants, no response was yet evident. Therefore, a soil drench method was used and the concentration of GA₃ also increased to ten times the maximum concentration applied as a spray.

1. Effects of GA₃ on the Diameter of Terminal Flowers of Carnation Plants Growing in CO₂ Enriched and Natural Atmospheres

The average diameter of terminal flowers of carnation plants growing in both CO₂ enriched and natural atmosphere was increased as a result of GA₃ treatment. Duncan's Multiple Range Test indicated that there were significant differences between treatments and the controls both in CO₂ enriched and natural atmosphere. The differences in diameter of terminal flowers are summarized in Table XX.

2. Effects of GA₃ on Internode Expansion of Terminal Stems of Carnation Plants Growing in CO₂ Enriched and Natural Atmosphere

In both CO₂ enriched and natural atmospheres there was a trend toward an increase in average expansion of the upper 5 internodes of terminal stems of carnation plants as a result

THE HISTORY OF THE

REIGN OF KING CHARLES THE FIRST

IN WHICH ARE CONTAINED THE MOST IMPORTANT
AND INTERESTING PARTS OF HIS REIGN

BOOK I

THE REIGN OF KING CHARLES THE FIRST

FROM HIS CORONATION TO HIS DEATH

IN THE YEAR OF HIS AGE SIXTY-ONE
AND OF HIS REIGN THE SIXTY-THIRD

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THE REIGN OF KING CHARLES THE FIRST

FROM HIS CORONATION TO HIS DEATH

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FROM HIS CORONATION TO HIS DEATH

IN THE YEAR OF HIS AGE SIXTY-ONE

TABLE XX

The influence of GA₃ on the diameter of terminal flowers of Dianthus caryophyllus (William Sim) plants growing in CO₂ enriched and natural atmospheres

A. Mean Diameter of Terminal Flower

Treatments	Mean Diameter in cm	
	CO ₂ Enriched Atmosphere	Natural Atmosphere
0 ppm GA ₃	7.175 ^b	7.525 ^b
50 ppm GA ₃	7.550 ^a	7.725 ^a
100 ppm GA ₃	7.675 ^a	7.750 ^a
500 ppm GA ₃	7.675 ^a	7.975 ^a
1000 ppm GA ₃	7.725 ^a	8.125 ^a

B. Analysis of Variance

Source	DF	MS	Mean Diameter	
			CO ₂ Enriched Atmosphere F	Natural Atmosphere F
Replications	3			
Treatments	4	0.202	3.406*	0.205
Error	12	0.0593		0.0705
				2.901 ^{N.S.}

*Significant at 5% level.

N.S. Not significant at 5% level.

Numbers in each column which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's Multiple Range Test.

of GA₃ treatment. However, Duncan's Multiple Range Test revealed that there were significant differences among treatments only in CO₂ enriched atmosphere. No significant differences were found among treatments in natural atmosphere, although they showed a trend toward increased stem length with GA₃ treatment. The differences in stem length are summarized in Table XXI.

3. Effects of GA₃ Treatment on Terminal Stem and Flower Weight (Upper 5 Internodes with Terminal Flower) of Carnation Plants Growing in CO₂ Enriched and Natural Atmospheres

The average terminal stem weight including the terminal flower of carnation plants growing in natural atmosphere was increased with GA₃ treatment. Duncan's Multiple Range Test showed that there were significant differences among treatments only in natural atmosphere. No significant differences were found in CO₂ enriched atmosphere although the terminal stem and flower weight showed an increase trend with increase in strength of GA₃ application. The differences in terminal stem weight of carnation plants are summarized in Table XXII.

4. Effects of GA₃ on Terminal Stem and Flower Weight (15 inches in Length) of Carnation Plants Growing in CO₂ Enriched and Natural Atmospheres

The average terminal stem weight (15 inches in length) with terminal flower of carnation plants growing in natural atmosphere was increased as a result of GA₃ treatment. Duncan's Multiple Range Test revealed that there were significant differences among treatments only in natural atmosphere, although

TABLE XXI

The influence of GA₃ on terminal stem internode length (upper 5 internodes) of Dianthus caryophyllus (William Sim) plants growing in CO₂ enriched and natural atmospheres

A. Mean Terminal Stem Length

Treatments	Mean Stem Length in cm	
	CO ₂ Enriched Atmosphere	Natural Atmosphere
0 ppm GA ₃	37.43 ^b	37.35 ^a
50 ppm GA ₃	38.81 ^{ab}	37.65 ^a
100 ppm GA ₃	38.92 ^{ab}	37.36 ^a
500 ppm GA ₃	40.31 ^a	39.05 ^a
1000 ppm GA ₃	41.12 ^a	39.65 ^a

B. Analysis of Variance

Source	DF	MS	Mean Stem Length	
			CO ₂ Enriched Atmosphere F	Natural Atmosphere F
Replications	3			
Treatments	4	8.049	3.966*	4.588
Error	12	2.029		1.910 ^{N.S.}
			2.402	

*Significant at 5% level.

N.S. Not significant at 5% level.

Numbers in each column which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's Multiple Range Test.

TABLE XXII

The influence of GA₃ on terminal stem and flower weight (upper 5 internodes with terminal flower) of Dianthus caryophyllus (William Sim) plants growing in CO₂ enriched and natural atmospheres

A. Mean Terminal Stem Weight

Treatments	Mean Terminal Stem Weight in gms	
	CO ₂ Enriched Atmosphere	Natural Atmosphere
0 ppm GA ₃	13.35 ^a	14.675 ^b
50 ppm GA ₃	13.38 ^a	14.850 ^b
100 ppm GA ₃	13.68 ^a	15.435 ^{ab}
500 ppm GA ₃	13.85 ^a	15.553 ^{ab}
1000 ppm GA ₃	14.30 ^a	16.250 ^a

B. Analysis of Variance

Source	DF	MS	Mean Terminal Stem Weight		
			CO ₂ Enriched Atmosphere F	MS	Natural Atmosphere F
Replications	3				
Treatments	4	0.611	0.522 ^{N.S.}	1.552	1.436 ^{N.S.}
Error	12	1.107		1.081	

N.S. Does not exceed the 5% level of significance.

Numbers in each column which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's Multiple Range Test.

Page 1

The first part of the report is a general introduction to the project. It describes the purpose of the study, the objectives, and the scope of the work. It also provides a brief overview of the methodology used in the research.

1. Introduction

Year	Month	Day	Time	Location
2000	Jan	1	10:00	Room 101
2000	Jan	2	10:00	Room 101
2000	Jan	3	10:00	Room 101
2000	Jan	4	10:00	Room 101
2000	Jan	5	10:00	Room 101

2. Methodology

Year	Month	Day	Time	Location
2000	Jan	1	10:00	Room 101
2000	Jan	2	10:00	Room 101
2000	Jan	3	10:00	Room 101
2000	Jan	4	10:00	Room 101
2000	Jan	5	10:00	Room 101

3. Results and Discussion

The results of the study show that there is a significant correlation between the variables studied. The data indicates that the proposed model is a good fit for the data, and the results are consistent with the theoretical expectations.

a similar trend was evident among treatments in CO₂ enriched atmosphere. The differences in terminal stem weight (15 inches in length) with terminal flower are summarized in Table XXIII.

5. Effects of GA₃ on Terminal Stem Strength of Carnation Plants Growing in CO₂ Enriched and Natural Atmospheres

The terminal stem strength of carnation plants growing in CO₂ enriched atmosphere was decreased as a result of GA₃ treatment. Duncan's Multiple Range Test revealed that there were significant differences among treatments only in CO₂ enriched atmosphere, although a similar trend was evident among treatments in natural atmosphere. The differences in stem strength of carnation plants are summarized in Table XXIV.

DISCUSSION

In these experiments the effects of GA₃ on plants growing in a CO₂ enriched atmosphere were not as marked as those effects on snapdragon plants under similar conditions. This is mainly due to the fact that the entire life cycle of the snapdragon plants was completed within the CO₂ enriched atmosphere period. The carnation being a slower growing crop did not come into flower production until after CO₂ enrichment had been discontinued. Carbon dioxide was added only during the period from November 15, 1965, to April 15, 1966, when little ventilation was required to control temperatures and natural light intensities were low. Gibberellic acid treatments were actually made after the period of CO₂ enrichment. Furthermore, differences in the size of the two greenhouse compartments used in

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TABLE XXIII

The influence of GA₃ on terminal stem weight (15 inches in length) with terminal flower of Dianthus caryophyllus (William Sim) plants growing in CO₂ enriched and natural atmospheres

A. Mean Terminal Stem Weight (15 inches in length)

Treatments	Mean Terminal Stem Weight in gm	
	CO ₂ Enriched Atmosphere	Natural Atmosphere
0 ppm GA ₃	12.93 ^a	14.60 ^b
50 ppm GA ₃	13.15 ^a	15.23 ^a
100 ppm GA ₃	13.40 ^a	15.65 ^a
500 ppm GA ₃	13.53 ^a	15.78 ^a
1000 ppm GA ₃	13.33 ^a	16.28 ^a

B. Analysis of Variance

Source	DF	MS	Mean Terminal Stem Weight	
			CO ₂ Enriched Atmosphere F	Natural Atmosphere F
Replications	3			
Treatments	4	0.218	0.178 ^{N.S.}	1.584
Error	12	1.225		0.836

N.S. Does not exceed the 5% level of significance.

Numbers in each column which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's Multiple Range Test.

I have the pleasure to acknowledge the receipt of your letter of the 11th inst. and in reply to inform you that the same has been forwarded to the proper authorities for their consideration.

Very respectfully,
Your obedient servant,

Statement of the Receipts and Disbursements of the		
Month	Year	Amount
Jan	1880	100.00
Feb	1880	200.00
Mar	1880	300.00
Apr	1880	400.00
May	1880	500.00
Jun	1880	600.00
Jul	1880	700.00
Aug	1880	800.00
Sep	1880	900.00
Oct	1880	1000.00
Nov	1880	1100.00
Dec	1880	1200.00

Witness my hand and seal this 1st day of January 1881.

Statement of the Receipts and Disbursements of the		
Month	Year	Amount
Jan	1881	1300.00
Feb	1881	1400.00
Mar	1881	1500.00
Apr	1881	1600.00
May	1881	1700.00
Jun	1881	1800.00
Jul	1881	1900.00
Aug	1881	2000.00
Sep	1881	2100.00
Oct	1881	2200.00
Nov	1881	2300.00
Dec	1881	2400.00

Witness my hand and seal this 1st day of January 1882.

I have the pleasure to acknowledge the receipt of your letter of the 11th inst. and in reply to inform you that the same has been forwarded to the proper authorities for their consideration.

TABLE XXIV

The influence of GA₃ on the terminal stem strength of Dianthus caryophyllus (William Sim) plants growing in CO₂ enriched and natural atmospheres

A. Mean Terminal Stem Strength

Treatments	Mean Stem Strength lb/stem	
	CO ₂ Enriched Atmosphere	Natural Atmosphere
0 ppm GA ₃	296.20 ^a	430.93 ^a
50 ppm GA ₃	250.00 ^b	426.75 ^a
100 ppm GA ₃	246.25 ^{bc}	359.85 ^a
500 ppm GA ₃	237.25 ^{bc}	350.93 ^a
1000 ppm GA ₃	217.15 ^c	351.88 ^a

B. Analysis of Variance

Source	DF	MS	Mean Stem Strength		
			CO ₂ Enriched Atmosphere F	MS	Natural Atmosphere F
Replications	3				
Treatments	4	3354.5	6.109**	6788.671	1.118 ^{N.S.}
Error	12	549.083		6070.474	

**Significant at 1% level.

N.S. Does not exceed the 5% level of significance.

Numbers in each column which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's Multiple Range Test.

The following table shows the results of the experiments conducted on the effect of the concentration of the solution on the rate of reaction. The results are given in the form of a table.

Experiment 1		
Concentration of solution (M)	Time taken for reaction to complete (s)	Rate of reaction (1/s)
0.1	120	0.0083
0.2	60	0.0167
0.3	40	0.0250
0.4	30	0.0333
0.5	24	0.0417

Experiment 2		
Concentration of solution (M)	Time taken for reaction to complete (s)	Rate of reaction (1/s)
0.1	120	0.0083
0.2	60	0.0167
0.3	40	0.0250
0.4	30	0.0333
0.5	24	0.0417

The results of the experiments show that the rate of reaction increases as the concentration of the solution increases. This is because there are more particles of the reactants in a given volume, so the chance of a collision between them is greater.

these experiments made day temperature control very difficult during the summer months. The compartment in which CO₂ levels had been increased often reached day temperatures of 10 and 15° F higher than those in the compartment in which CO₂ levels had not been increased.

The average diameter of terminal flowers was increased as a result of GA₃ treatment both in CO₂ enriched and natural atmosphere. Although the methods of application of GA₃ may differ in these experiments, the results agree with those of Cathey (14), Davis, et. al. (20) and Lindstone and Wittwer (37). This phenomenon of flower size increase may be due to an increase in petal size (14, 20, 32) brought about by increased cell division and cell elongation (42, 47).

The terminal stem length was increased as a result of GA₃ treatment under natural atmosphere conditions. The most typical response of plants to GA₃ of course, is stem elongation (14, 15, 42). Elongation results primarily from an increase in the length of internodes (14). This is mainly due to increased cell length (55).

The terminal stem weight (both 5 internodes in length and 15 inches in length) with terminal flower was increased with GA₃ treatment. These results support the hypothesis which was made by Hayashi (28) who suggests that the photosynthetic activity per unit leaf area does not change as a result of gibberellic acid treatment, but owing to the increase in leaf area, the photosynthetic activity of the whole plant increases. Such overall increase in photosynthesis results in increased

weight. It has also been reported by Brian (6) that under some circumstances, with some plant species, treatment with GA_3 results in increased weight. This is mainly due to the increase in photosynthetic activity and is believed to be a secondary effect of increased leaf growth. When we compare the control plants both in CO_2 enriched and natural atmospheres, we find a higher percentage increase in weight in plants in natural atmosphere. These rather unexpected results may be due to the higher temperature during the summer months in the compartment in which CO_2 enrichment had been made during the previous winter. During the summer months the day temperature in this compartment often went above $100^{\circ} F$ regardless of the fact that all the ventilators were open and the roof shaded. Within certain limits, increase in temperature results in an increase in the respiration rate. If the temperature is very high, such as over $100^{\circ} F$, the photosynthetic activity will decrease but the respiration rate will continue to increase as the temperature goes up (4). Under the conditions of long days and high temperatures there will be a more rapid breakdown than buildup of carbohydrates and consequently the weight of terminal stems and flowers will decrease.

The stem strength of the plants raised in CO_2 enriched atmosphere was decreased as a result of GA_3 treatment. Again these results are in keeping with the hypothesis which was made earlier in the discussion of the chrysanthemum experiments. When we compare the effect of GA_3 both in CO_2 enriched and natural atmosphere, the greater and only significant effects on stem strength were found under CO_2 enriched atmosphere

conditions. The differences in results in CO₂ enriched and natural atmosphere may be due to the temperature differential rather than to the differences in CO₂ levels.

These results support the hypothesis which was made by Stuart and Cathey (57) who suggest that the growth of a plant is a net result of the influence of growth promoting materials versus growth inhibiting materials. It has been observed that gibberellin often induces maximum responses when growth is adversely influenced by temperature, nutrition or other environmental factors. Possibly under these conditions synthesis of both native gibberellin and inhibitor is retarded, permitting the greater response from exogenous gibberellin.

The following is a list of the names of the persons who have been elected to the office of the President of the United States, and the names of the persons who have been elected to the office of the Vice President of the United States, for the year 1856.

The names of the persons who have been elected to the office of the President of the United States are: James Buchanan, Franklin Pierce, and John Fremont.

The names of the persons who have been elected to the office of the Vice President of the United States are: William A. R. Harris, William A. R. Harris, and William A. R. Harris.

VI. SUMMARY AND CONCLUSION

This investigation was conducted to determine the effect of gibberellic acid on the growth and flowering of Chrysanthemum morifolium, Antirrhinum majus and Dianthus caryophyllus plants under special greenhouse conditions.

1. The terminal stem length of chrysanthemum plants both in short and long photoperiod regimes was increased as a result of GA₃ treatment but more marked under short photoperiod.
2. The stem strength of chrysanthemum plants both in short and long photoperiod regimes was decreased with GA₃ treatment.
3. The terminal stem fresh weight of chrysanthemum plants both in long and short photoperiod regimes was increased as a result of GA₃ treatment, but more markedly in long photoperiod.
4. The number of internodes of terminal stems of chrysanthemum plants both in long and short photoperiod regimes was increased with GA₃ treatment but more markedly in long photoperiod.
5. The leaf number of terminal stems of chrysanthemum plants both in short and long photoperiod regimes was increased as a result of GA₃ treatment but more markedly in long photoperiod.
6. The leaf fresh weight of terminal stem leaves of chrysanthemum plants was increased only in short photoperiod regime.

7. The root weight of chrysanthemum plants both in long and short photoperiod was decreased as a result of GA₃ treatment.
8. The dry weight of the terminal stems of chrysanthemum plants both in short and long photoperiod was increased with GA₃ treatment but more markedly in long photoperiod.
9. The dry weight of leaves of chrysanthemum plants both in short and long photoperiod regimes was increased as a result of GA₃ treatment.
10. The fresh weight and diameter of terminal inflorescences of chrysanthemum plants were increased with GA₃ treatment under the short photoperiod regime. No flower bud initiation and development occurred under long photoperiods regardless of GA₃ treatment.
11. The dry weight of terminal inflorescences and the length of the 1st lateral peduncles of chrysanthemum plants were increased with GA₃ treatment under short photoperiods.
12. The average chlorophyll (a + b) content in leaves of chrysanthemum plants was decreased as a result of GA₃ treatment but differences were significant only under the long photoperiod regime.
13. The inflorescence length of snapdragon plants in CO₂ enriched and natural atmosphere was increased as a result of GA₃ treatment. However, differences were significant only under the long photoperiod regime.
14. The stem length of snapdragon plants both in CO₂ enriched and natural atmosphere was significantly increased with GA₃ treatment.

15. The fresh weight of tops of snapdragon plants in CO₂ enriched and natural atmosphere was increased with GA₃ treatment but differences were significant only under the long photoperiod regime.
16. The root length of snapdragon plants both in CO₂ enriched and natural atmosphere was slightly inhibited but differences were significant.
17. The chlorophyll (a + b) content in leaves of snapdragon plants both in CO₂ enriched and natural atmosphere was significantly decreased as a result of GA₃ treatment.
18. The diameter of terminal flowers of carnation plants growing in both CO₂ enriched (winter months only) and natural atmospheres was increased as a result of GA₃ treatment but more marked under natural atmosphere.
19. The length of terminal stems of carnation plants in CO₂ enriched atmosphere only was increased as a result of GA₃ treatment.
20. The terminal stem weight (both 5 internodes and 15 inches in length) with terminal flower was increased as a result of GA₃ treatment in natural atmosphere only.
21. The terminal stem strength of carnation plants growing in CO₂ enriched atmosphere was decreased with GA₃ treatment.

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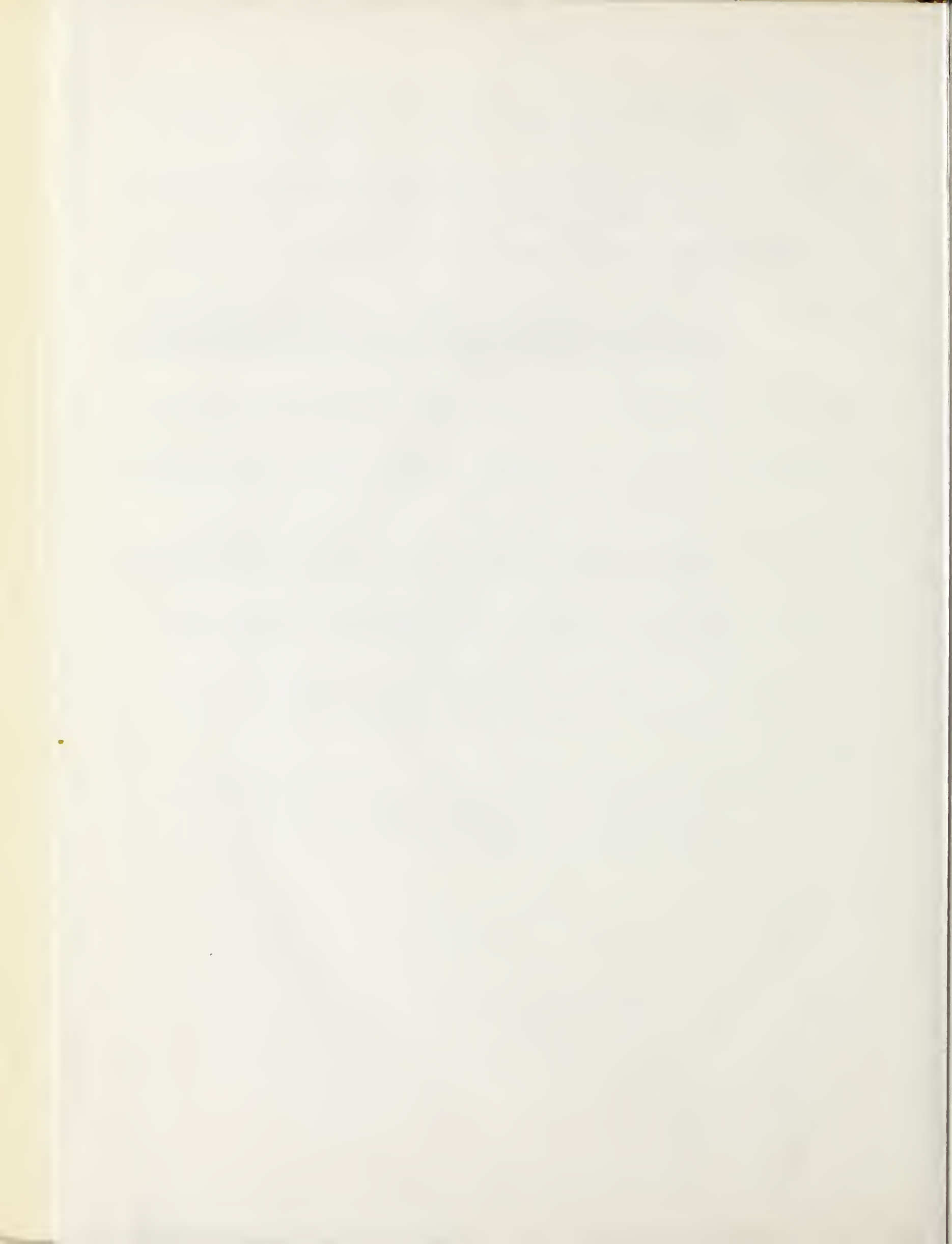
ADDENDUM

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1. The first of these is the fact that the number of cases of the disease has increased in the last few years.
2. The second is the fact that the disease is now found in many more countries than it was a few years ago.
3. The third is the fact that the disease is now found in many more parts of the world than it was a few years ago.
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10. The tenth is the fact that the disease is now found in many more parts of the world than it was a few years ago.

It is clear from the above that the disease is now found in many more parts of the world than it was a few years ago.





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